C H A P T E R - 5

DISCUSSION OF THE EXPERIMENTAL RESULTS ON EXPERIMENTAL EVALUATION OF ALGAL SYMBIOSIS:

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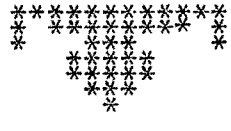


CHAPTER V

(A) Bio-oxidation of decomposing organic matter:

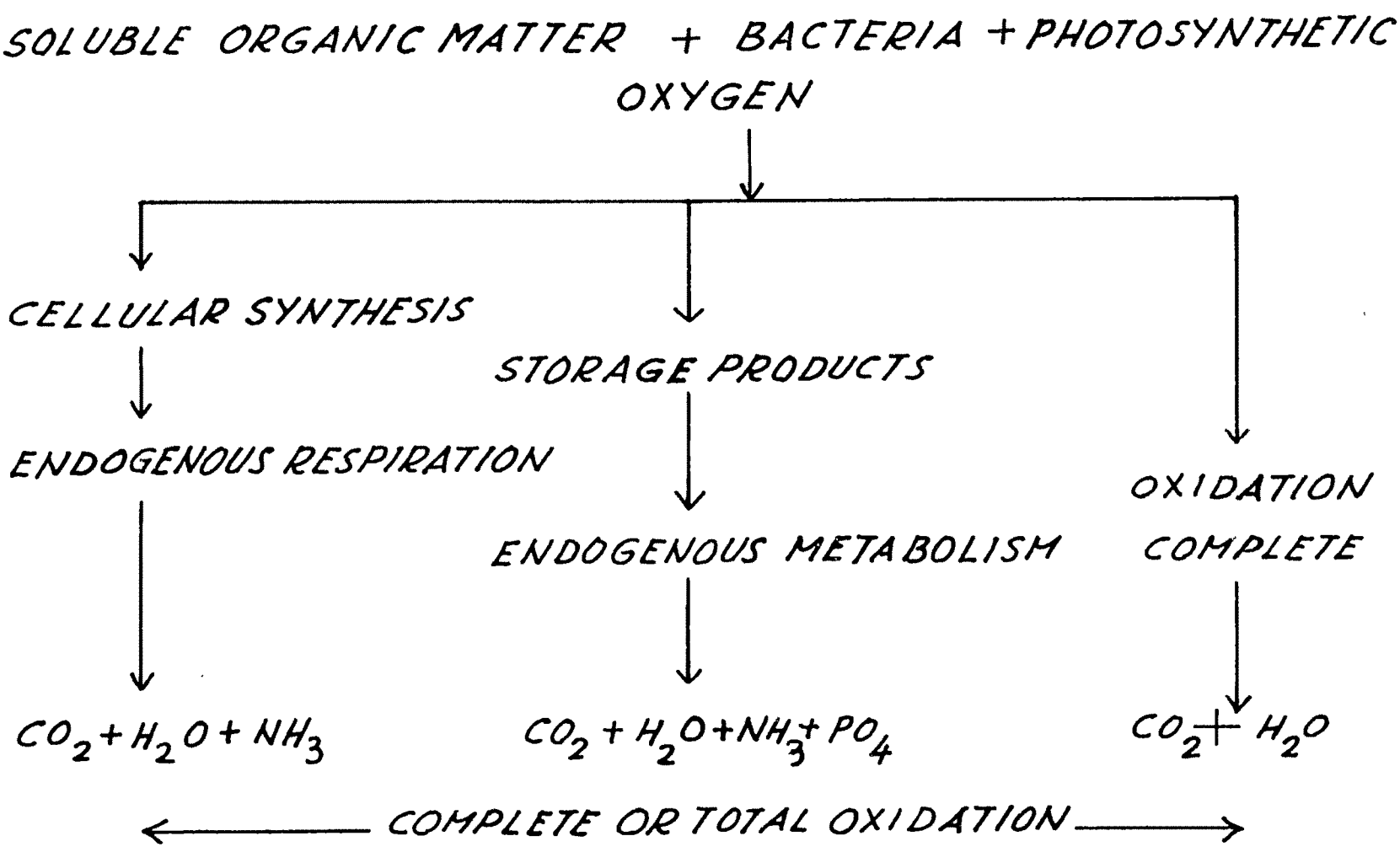
1. Four important principles of bio-oxidation of organic matter are concerned in waste treatment system.

   (a) The phenomenon of mechanical flocculation, bio-flocculation, bio-precipitation and surface aeration:

The phenomena of mechanical flocculation, bio-flocculation and bio-precipitation are of common occurrence in nature and act as partial and intermediary processes of sewage purification (Heukelekian 1941). The process of flocculation is coalescence of finely divided suspended matter in sewage acting in the absence of biologically active slime, primarily under the influence of physical forces. Bio-flocculation is also coalescence of finely divided suspended matter but acting under the influence of biological agencies. The conditions favouring this type of reaction are long periods of either quiescence or agitation without the addition of biologically active slime. Bio-precipitation is distinguished from bio-flocculation by the conversion of soluble substrates

FIG. 2

COMPLETE OR TOTAL BACTERIAL OXIDATION OF ORGANIC MATTER (MODIFIED AFTER PORGES 1960]



into cellular protoplasm by micro-organisms. This is further explained later. The design criteria and the method of operation in high-rate aerobic ponds are conducive to foster these phenomena which seem to act as partial and intermediate process of sewage purification.

Surface aeration is another factor which plays an equally important role especially in the nights when photosynthesis is impossible. It helps to drive oxygen from the air into the liquid as in the case of the Simplex Activated Sludge Process. The wind action across the huge pond surface in the shallow ponds causes the breaking of the liquid surface and mixing of the pond contents. Thus it helps to keep the system aerobic.

(b) Complete Oxidation:

Complete oxidation of organic compounds by bacteria resulting in the formation of carbon-dioxide and H_2O as the final degradation products, of metabolism where a great number of enzymes and intermediate products are involved besides the electron transfer.

Organic matter present in sewage as suspended or colloidal solids is removed by coagulation,

entrainment, adsorption and oxidation of components made soluble by enzymes. These reactions are illustrated by the following equation:-

(i) Organic matter, complete oxidation

$$C_xHyO_z + (x + \tfrac{1}{4}y - \tfrac{1}{2}z)\ O_2 \longrightarrow$$

$$x\ CO_2 + \tfrac{1}{2}y\ H_2O + \Delta H \quad \ldots\ldots\ldots\ldots\ (1)$$

Equation (1) is the conventional equation of combustion. If nitrogen is present, it will be oxidized to nitrate, sulfur will be oxidized to sulfate. This is the case for complete oxidation of organic matter.

(c) Incomplete Oxidation:

Biosynthesis and growth accompany the decomposition of organic substrates and newly formed cells are major end products of intermediate metabolism. This process is also known as "Cellular Synthesis" and "Oxidative assimilation". The patterns of substrate utilization may be either concurrent or sequential. In incomplete oxidation where conversion of an organic substrate to carbondioxide and water does not take place but oxidized organic compounds accumulate in the medium as end products of respiratory metabolism. The reactions of the acetic acid bacteria are classical examples.

(ii) Cell material synthesis:

$$n(C_xH_yO_2) + nNH_3 + n(x + \tfrac{1}{4}y - \tfrac{1}{2}z - 5)\, O_2 \longrightarrow$$
$$(C_5H_7NO_2)n + n(x-5)\, CO_2 + \tfrac{1}{2}n\,(y - 4)\, H_2O - \Delta H \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

This equation represents the stage of cellular synthesis. The synthesis of activated sludge is shown by equation No.(2) employing ammonia as a source of nitrogen. The cellular material is represented by the emperical formula $C_5H_7NO_2$ developed by Hoover. This formula is representative of the ratio of primary constituents of activated sludge. It is representative of the statistical average composition of the complex organic compounds constituting the cell material.

(d) Endogenous metabolism:

Endogenous metabolism takes place when the organic material in solution is very low so that bacteria derive energy from the destruction of their own storage products. At this stage the number of active organism is low and most bacteria then lose their motility (McKinney, 1956). Again according to Mckinney (1952, 1956) bacterial flocculation takes place when the micro-organisms are in a state of endogenous respiration in treatment systems.

(iii) Cell material Oxidation:

$$(C_5\text{-}H_7NO_2)\ n + 5nO_2 \rightarrow 5nCO_2 + 2nH_2O + nNH_3 - \Delta H \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

This equation represents endogenous respiration of bacterial cells or the complete oxidation of cellular material previously synthesized (Fig. 2).

The factors x, y, and z in the above equations may be positive or zero depending upon the type of compound involved. The term H represents the heat of reaction. These generalized equation may be modified for organic compounds containing nitrogen or sulphur.

(2.) Biochemical assessment of viable bacteria and related results in growth cycles of algal-bacterial symbiosis with the two algae Oscillatoria spp. and Anacystis nidulans:

The main purpose of aerobic biological treatment for stabilization of organic matter is the removal of organic substances of the waste water. The removal is brought about by reactions emboided in the two cardinal principles of complete oxidation and oxidative assimilation that the activated sludge process maintains and even increases itself (Symons and Mckinney, 1958).

These two biochemical reactions - complete oxidation and oxidative assimilation - may be thus expressed:-

Total organic matter removed in waste water = bacterial oxidation + cellular synthesis.

It will be evident from the above equation that if the process of cellular synthesis of bacterial cells is to be reduced in any aerobic waste treatment system, then an increase in bacterial oxidation process must take place should the sum total of organic matter removed is to remain the same.

So it will be evident that a very striking feature of microbial metabolism in waste water treatment systems is the relatively enormous amount of new bacterial cells that is normally produced during the breakdown of organic matter. Rapid purification of a waste depends upon the unrestricted activities and reproduction of micro-organisms (Sawyer 1956).

i) Removal of organic pollutants as COD:

Respiration is an indespensible process of living creatures. For this process organisms demand oxygen from the environment. Thus sewage exerts a BOD i.e. biological oxygen demand

through the organisms living on it; so that these organisms can oxidise organic matter.

BOD estimates the oxygen demand by bacteria for the 5 days period only while chemical oxygen demand(COD)estimates oxygen demand exerted by a bacteria for their life span i.e. 20 days.

Reduction in COD values represents indirectly the organic matter removed by bacteria i.e. COD values indirectly give an idea of the amount of organic matter present in sewage. It gives an opportunity to express one important statement of Porges. Porges has stated: "The COD tests proved indispensable in his laboratory investigations" (Porges 1960, p. 9). Percentage reduction of chemical oxygen demand in the two algae treated experiments is summarised below:

Percentage Reduction of COD

Detention period	Control raw sewage	Oscillatoria spp.	Anacystis nidulans
2 days	20 to 28	68.8	65
4 days	39 to 46	84.9	80
6 days	64 to 66	90.3	92.34

The organic matter removed in control within six days is 64 to 66 percent while in the algae treated two experiments the same amount was removed within two days. The percentage removal of organic matter by the two algae in six days is 90 to 92.

The reduction of chemical oxygen demand in control flasks has to be attributed mainly to the phenomena of mechanical flucculation, bio-flocculation, bio-precipitation and surface aeration which are of common occurrence in nature (Heukelekian 1941, Oswald, 1960). The increased percentage reduction of COD has to be ascribed to photosynthetic oxygen furnished to bacteria as a result of algal-bacterial reactions in the algal growth cultures.

(ii) Calculations of bacterial growth and synthesis according to Sawyer (1956):

Nutrition and synthesis are immediately related factors. Synthesis and growth are essentially synonymous terms. Growth results from the marshalling of part of food supply and accessory mineral factors to produce new generations of the organisms with characteristic and chemical composition similar to the progeniters. A considerable fraction of the food is

used for energy purposes. Gellman and Heukelekian (1953), Hoover and Porges (1952) and Weston and Eckenfelder (1956) have found that the yield of cell material during the rapid growth phase is slightly over 50% of the carbon utilized and the remaining half is completely oxidized to CO_2, H_2O and energy in aerobic treatment systems. Sawyer (1956) and Mckinney (1962) have furnished two different methods for calculating the cellular biomass which represents only about 50% of the carbon utilized. Oswald et al. (1958) on the other hand has furnished a formula for complete oxidation of sewage organic matter resulting in CO_2 and water and ammonia. All the three methods of calculation are shown under

Growth and synthesis can be predicted from 5-day BOD of a waste. Sawyer (1956) has found a formula to calculate total bacterial mass in activated sludge process which is given below:

Total bacterial growth = 0.5 x BOD_5 used up. The same formula has been applied in our case also.

(a) <u>Total bacterial mass in the case of control (raw sewage used for Oscillatoria spp. and Anacystis nidulans)</u>.

Detention period in days	Raw sewage used for Oscillatoria Experiment		Raw sewage used for Anacystis nidulans Experiment	
	BOD_5 used up	Total mass	BOD_5 used up	Total mass
2	89	44.5	41	20.5
4	175	87.5	58	29.0
6	207	103.5	108	54.0

(b) Total bacterial mass in algal-treated samples:

Detention period in days	Oscillatoria ssp. (mg/l)		Anacystis nidulans (mg/l)	
	BOD_5 used up	Total mass	BOD_5 used up	Total mass
2	230	115.0	102	51.0
4	255	127.5	131	65.5
6	260	130.0	152	76.0

Bacterial mass formed in the case of the algal-treated samples is considerably higher than in the case of control samples.

(iii) Calculation of actual bacterial biomass and decrease in active biomass due to endogenous metabolism according to Mckinney (1962).

"Micro-organism can continuously remove organic matter from liquid waste by synthesis into new protoplasm. It is possible for the micro-organisms to absorb quantities of organic matter into their cell surfaces but unless this absorbed organic matter is assimilated into protoplasm the rate of absorption will approach zero. Since a definite quantity of organic matter is required to form the building blocks for the microbial cells and a definite quantity of organic matter must be oxidized to form the energy necessary for synthesis, a relationship exists between the removal of organic matter and the cells synthesized together with the oxygen consumed".

"The aerobic systems are the primary systems for waste treatment and include activated sludge, trickling filters, oxidation ponds and composting. The basic equation is expressed directly in terms of dissolved oxygen uptake as long as the systems remain truely aerobic. Since there is a definite quantity of energy required to produce a definite quantity of protoplasm, an equation between the synthesis and energy can be established".

Mckinney (1962) has found a formula which expresses the removal of organic matter in terms of synthesis in the case of activated sludge process. The same has been applied in our case also and the results are shown below:

$F = 2.13 \times S$ Where F = Organic matter removed, mg/litre ultimate BOD; S = Synthesis, mg/litre and volatile solids i.e. total bacterial mass.

(a) Calculations for the control of Raw sewage samples used in the two algal experiments are shown under:-

Detention period in days	Oscillatoria spp. (mg/l) COD used up	Oscillatoria spp. (mg/l) Total mass	Anacystis nidulans (mg/l) COD used up	Anacystis nidulans (mg/l) Total mass
2	78	36.62	80	37.56
4	172	80.84	110	51.64
6	240	112.67	185	86.85

(b) Calculations for the algal-treated samples are given below:

Detention period in days	Oscillatoria spp. (mg/l) COD used up	Total mass	Anacystis nidulans (mg/l) COD used up	Total mass
2	256	102.2	182	85.44
4	316	148.35	224	105.16
6	336	157.74	258	121.12

The total bacterial mass in the two algae-treated samples are considerably more than in the respective controls of raw sewage only.

Mckinney has stated : "The problem remaining is to determine exactly what is synthesis. It is not a direct measurement of the increase in mass but rather is the sum of the increase in active mass plus decrease in active mass due to endogenous metabolism." The equation is as follows:

$S = Ma + K_3 Mat.$

Where: Ma = active microbial mass, mg/litre of volatile solids,

*t = time in hour, $K_3 = 0.006$, and S = synthesis/mg/l of total bacterial mass.

Endogenous bacterial mass = Total bacterial mass — active bacterial mass.

(c) Calculations of total bacterial mass, active bacterial mass and endogenous bacterial mass are shown below in a tabular form. The results are expressed in mg/l.

In the two algae-treated samples:

Detention period in days	Oscillatoria spp. (mg/l) Total mass	Active mass	Endog. mass	Anacystis nidulans (mg/l) Total mass	Active mass	Endog. mass
2	102.2	79.35	22.85	85.44	66.34	19.1
4	148.35	94.13	54.22	105.16	66.72	38.4
6	157.74	84.62	73.12	121.12	65.0	53.1

The percentage of endogenous bacterial mass would seem to range from 22.0% to 46% during 2 to 6 days of the detention periods in the case of the two algae.

The data indicate plainly that the total bacterial mass calculated according to Sawyer's equation are less than the total bacterial mass calculated according to Mckinney. But it would appear that they seem to be related more to the active mass of Mckinney. So an attempt is made to correlate the total bacterial mass

of Sawyer with active bacterial mass of Mckinney below, assuming that the metabolic activities of bacteria are similar to an activated sludge process in the high-rate pond. The results are expressed in mg/l.

Detention period in days	Oscillatoria spp. (mg/l) Total mass (Sawyer)	Active mass (Mckinney)	% Diff.	Anacystis nidulans (mg/l) Total mass (Sawyer)	Active mass (Mckinney)	% Diff.
2	115.0	79.35	-31.0	51.0	66.34	+31.0
4	127.5	94.13	-26.17	65.5	66.72	+ 1.86
6	130.0	84.62	-34.91	76.0	65.00	-14.49

It has to be borne in mind that the formulae of Sawyer and Mckinney are related to activated sludge process and not to the high-rate aerobic oxidation ponds. The data with Anacystis nidulans show that total mass calculated according to Sawyer is similar to the active mass calculated according to Mckinney. It is approximately the same but in the case of Oscillatoria spp. Calculated active mass is about two thirds of the calculated total bacterial mass by Sawyer's formula. So, the results seem to vary in the two case.

(d) Quantitative evaluation of Algal-bacterial symbiosis in the case of the two algal types experimented with Baroda raw, strained sewage.

In order to evaluate algal-bacterial symbiosis (or how one helps the other) in our experiments with the two different types of algae, it is necessary to know the quantity of CO_2 liberated during total bio-oxidation of sewage organic matter for the production of each of the two algal biomasses, and in turn also how much of the photosynthetic O_2 is liberated by each of the algal biomasses during photosynthesis for total bacterial oxidation of sewage organic matter. But it is not possible to estimate directly either of the two gases in the ecosystems during algal-bacterial symbiosis, for, they are not phased metabolic processes (i.e. one taking place after the other), but they are considered to be not only almost simultaneous or concurrent, but are also stated to be utilized as soon as they are liberated in the ecosystem (Oswald, 1960). The two metabolic processes are illustrated in Fig. I.

So, attempts were made to estimate the quantity of the two gases indirectly by methods which are based upon certain well-established factors and equations

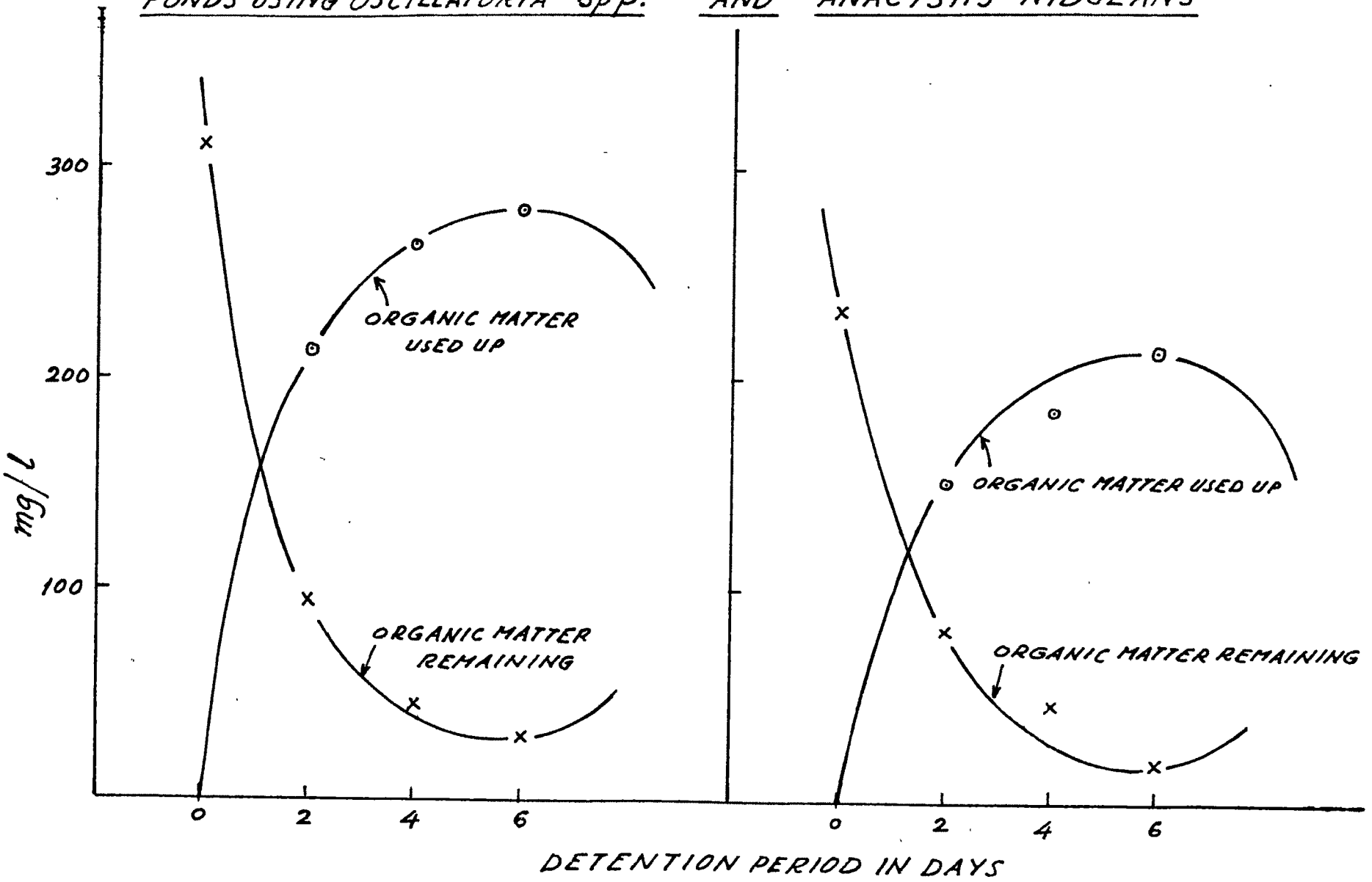
connecting CO_2 production from and oxygen requirements for total oxidation of sewage organic matter, and photosynthetic oxygen production from algal biomasses formed. This is the first time that such an attempt has been made in the history of the oxidation pond literature for establishing new relations of facts from the two most indispensable and important parameters-COD and algal biomass - which have been actually determined in our laboratory experiments.

"COD" as we know, is a measure of the quantity of the oxygen required for total bio-oxidation of sewage organic matter and not of the organic matter itself. But we have to know the quantity of organic matter oxidized by bacteria; and Porges (1960) has furnished a method of estimating approximately the same from COD values by using the conversion factor 1.2. COD values when divided by 1.2 gms. the corresponding "organic matter" equivalent in waste water. The rest of the calculations are shown below:

(iv) Conversion of COD values into organic matter values according to Porges (1960): (Fig. 3).

FIG. 3

BIO-OXIDATION OF SEWAGE ORGANIC MATTER IN HIGH-RATE AEROBIC OXIDATION PONDS USING OSCILLATORIA spp. AND ANACYSTIS NIDULANS



The quantity of oxygen needed for the total oxidation of sewage organic matter has been worked out using first the conversion of COD values into organic matter. The factor of 1.2 has been used for converting COD values into corresponding organic matter according to Porges 1960 (pages 6 & 20).

Detention period in days	Oscillatoria spp. + Raw sewage (mg/l) COD	COD used up	Anacystis nidulans + Raw sewage (mg/l) COD	COD used up
0	372	-	280	-
2	116	256	98	182
4	56	316	56	224
6	36	336	22	258

Conversion of COD values into organic matter values

Detention period in days	Oscillatoria spp. (mg/l) COD used up	Organic matter	Anacystis nidulans (mg/l) COD used up	Organic matter
2	256	213.3	182	152
4	316	263.3	224	187
6	336	280.0	258	215

FIG. 4

BACTERIAL OXIDATION OF ORGANIC MATTER

USING OSCILLATORIA Spp.

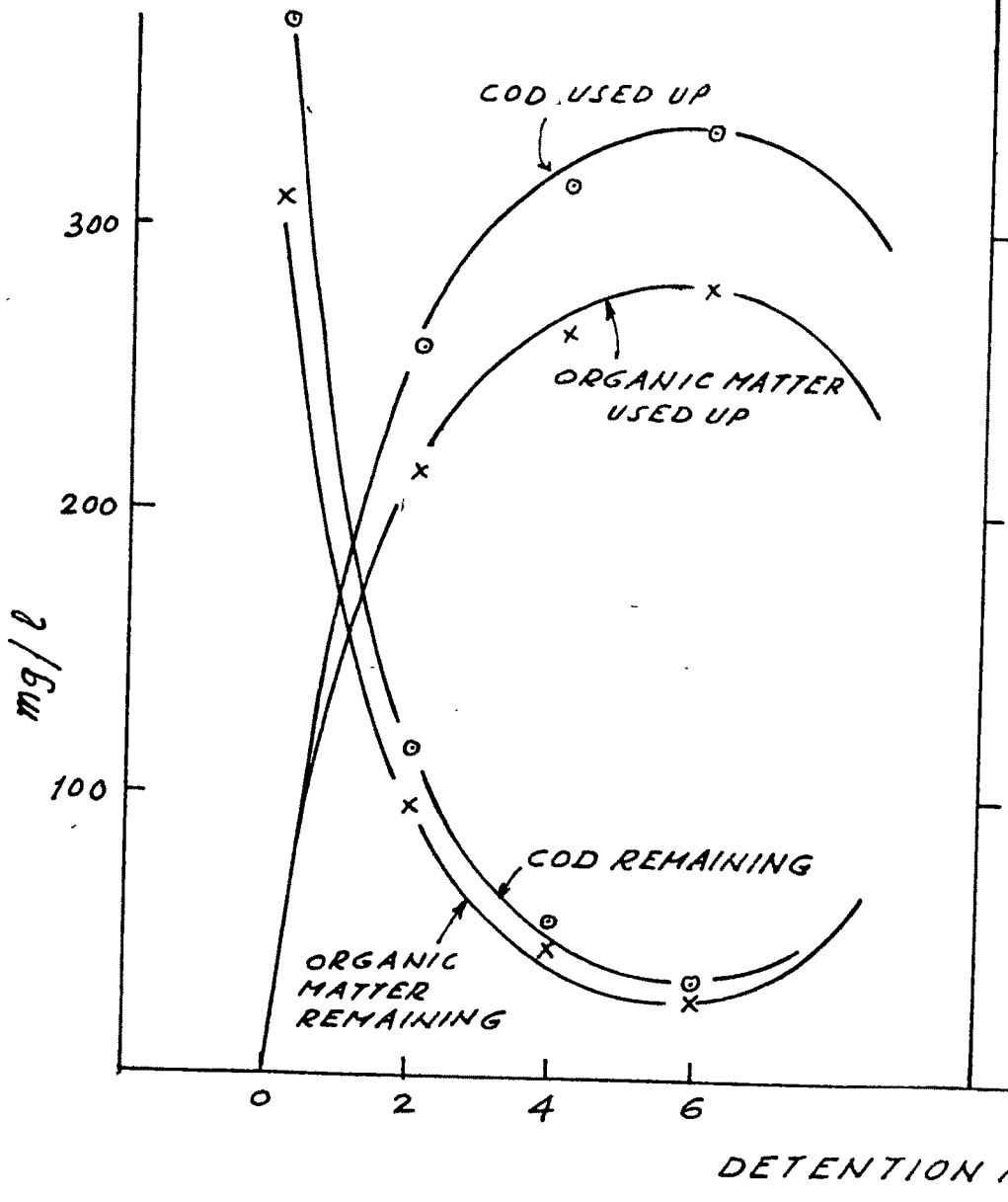
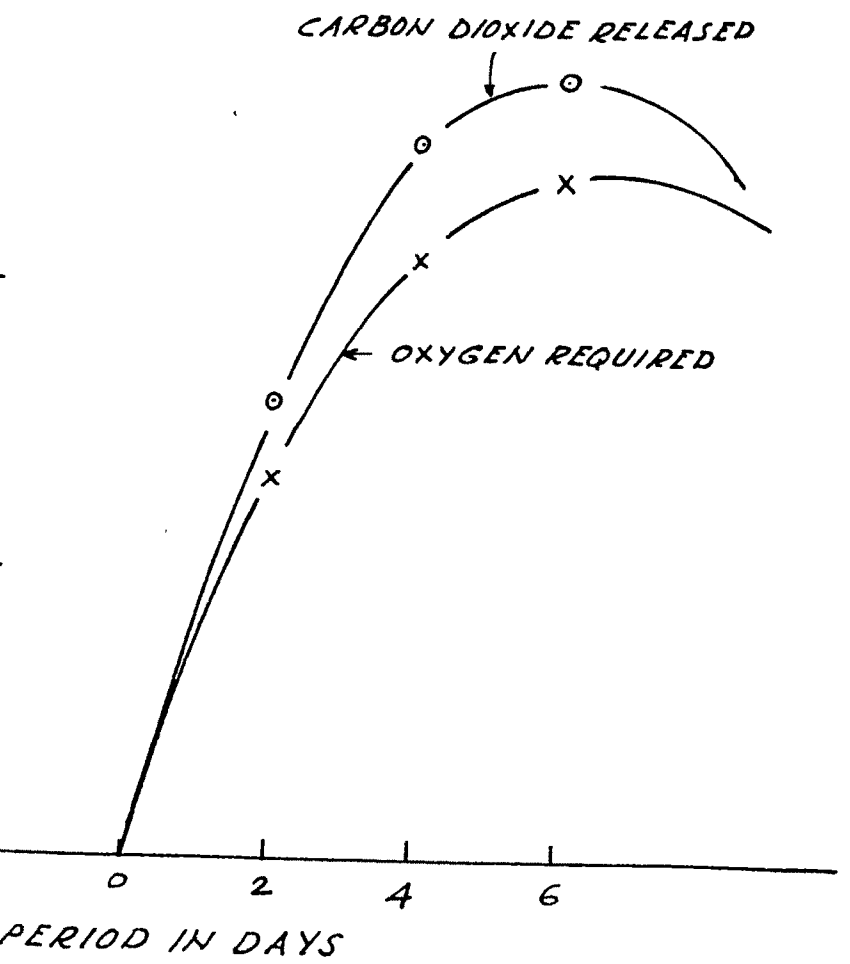


FIG. 5

TOTAL BIO-OXIDATION OF ORGANIC MATTER

USING OSCILLATORIA Spp.



DETENTION PERIOD IN DAYS

FIG. 6

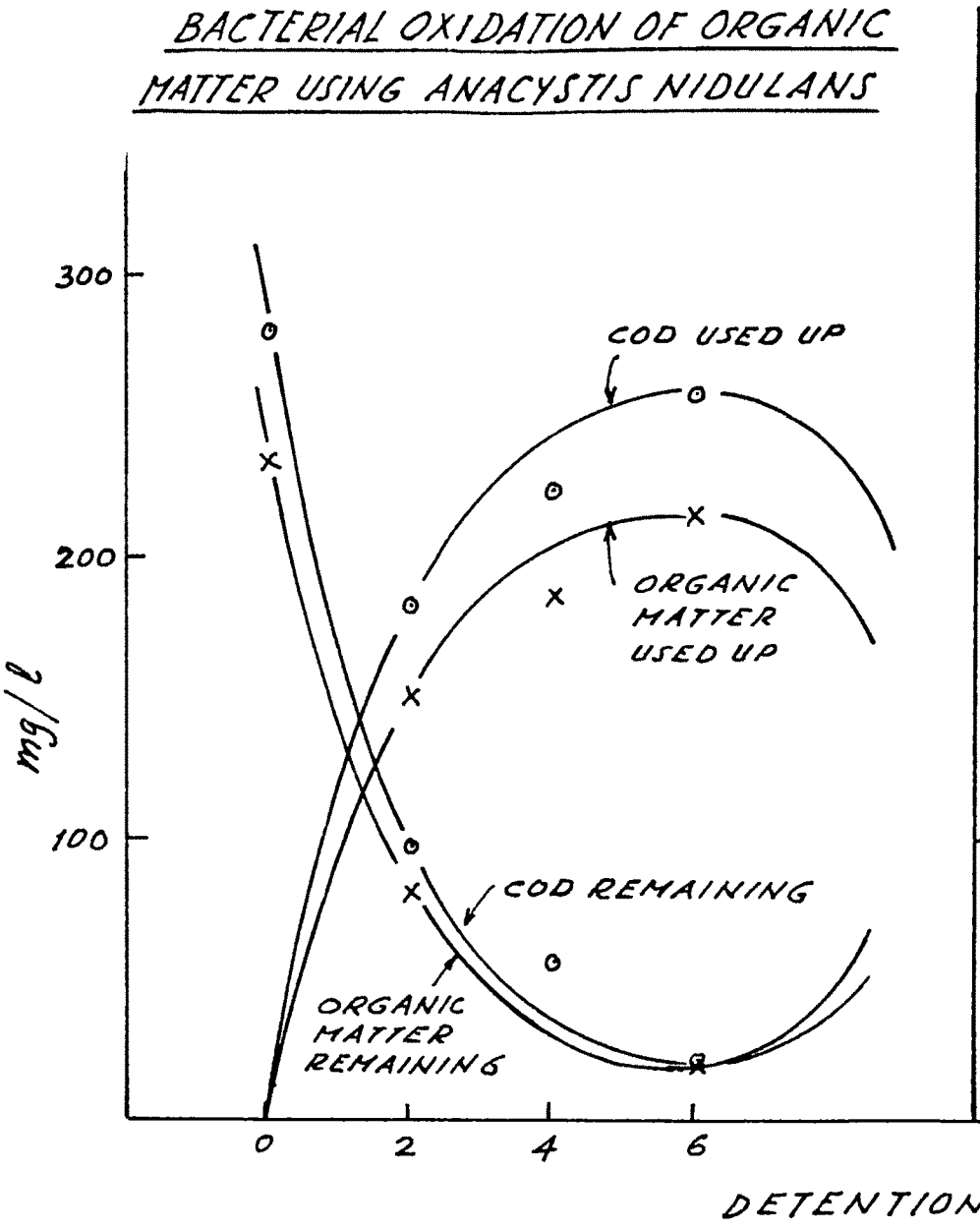
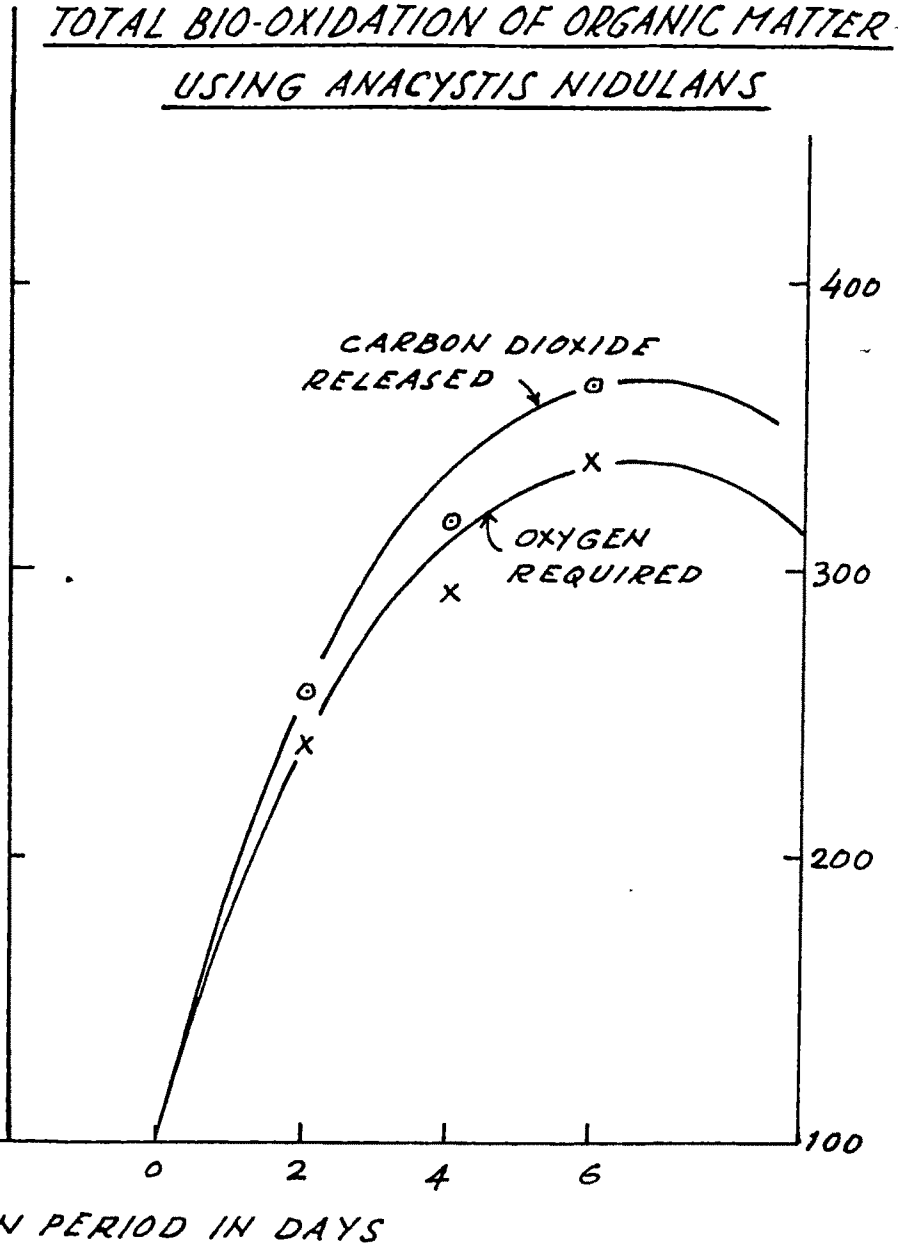

BACTERIAL OXIDATION OF ORGANIC MATTER USING ANACYSTIS NIDULANS
300
200
100
mg/l
COD USED UP
ORGANIC MATTER USED UP
COD REMAINING
ORGANIC MATTER REMAINING
0
2
4
6

FIG. 7


TOTAL BIO-OXIDATION OF ORGANIC MATTER USING ANACYSTIS NIDULANS
400
300
200
100
CARBON DIOXIDE RELEASED
OXYGEN REQUIRED
0
2
4
6
DETENTION PERIOD IN DAYS

(v) Calculation of Oxygen required and carbon dioxide released during bacterial oxidation of Baroda sewage organic matter according to Oswald et al. (1958).

Oswald et al. (1958) found experimentally in high-rate aerobic ponds, the oxidation of organic matter to follow the equation:

$$C_{11}H_{29}O_7N + 14O_2 + H \longrightarrow 11CO_2 + 13H_2O + NH_4$$

organic matter 287 gms + 448gm O_2 --> 484CO_2 + 13H_2O + NH_4

So, 1gm of sewage organic matter will produce 1.69gm carbon dioxide and will require 1.56gm of oxygen for complete oxidation.

Calculation of the quantity of oxygen required for the total bacterial oxidation of organic matter(Oxygen required) = Organic matter x 1.56.

The results in our case are shown below:

Detention period in days	Oscillatoria Spp. (mg/l) Organic matter used up	Oscillatoria Spp. (mg/l) Oxygen requirement	Anacystis nidulans (mg/l) Organic matter used up	Anacystis nidulans (mg/l) Oxygen requirement
2	213.3	332.7	152	237.1
4	263.3	410.7	187	291.7
6	280.0	436.8	215	335.4

Next, the quantity of carbon dioxide released during bacterial oxidation of organic matter will then be organic matter x 1.69.

The results in our case are shown below:

Detention period in days	Oscillatoria spp.(mg/l) Organic matter used up	Carbon dioxide released	Anacystis nidulans(mg/l) Organic matter used up	Carbon dioxide released
2	213.3	360.5	152	256.9
4	263.3	445.0	187	316.0
6	280.0	473.2	215	363.3

It has to be found out whether the quantity of oxygen required for bacterial oxidation of organic matter and the resulting carbondioxide released therefrom are sufficient, the former for the algal photosynthesis and the latter for bacterial oxidation (Fig. 4 to 7).

(vi) Oxygenation factor:

Oswald (1964) has established a criterian called the "Oxygenation factor" for the successful working of an high-rate aerobic oxidation pond. It is a ratio which should be above 1.0 when the photosynthetic oxygen produced through algal activity is divided by the amount of oxygen required to meet BOD_5 of the influent

sewage. Values for the oxygenation factors for the several days of detention period are shown in the tabular statement for the three experiments with three algal specimens. COD values also have been taken into account for calculating oxygenation factor shown in the table below:

Name of algae	Detention period in days	Photosynthetic O_2	COD mg/l Found	COD mg/l Used	BOD_5 mg/l Found	BOD_5 mg/l Used	Ratio = Photo synthetic O_2 / COD	Ratio = Photo synthetic O_2 / BOD_5
Oscillatoria Spp.								
	0	--	372	-	280	-	-	-
	2	460.8	116	256	50	230	1.8	2.0
	4	483.2	56	316	25	255	1.5	1.9
	6	496.0	36	336	20	260	1.5	1.9
Final factor (average)			-	-	-	-	1.33	1.
Anacystis nidulans								
	0	--	280	-	166	-	-	-
	2	307.2	98	182	64	102	1.6	3.0
	4	320.0	56	224	35	131	1.4	2.4
	6	326.0	22	258	14	152	1.3	2.1
Final factor (average)			-	-	-	-	1.56	-
Scenedesmus obliqus								
	0	--	408	-	-	-	-	-
	2	396.8	144	264	--	-	1.5	-
	4	449.6	64	344	-	-	1.3	-
	6	457.6	46	362	-	-	1.26	-
Final factor (average)			-	-	-	-	1.1	-

It will be evident from the table that the ratios are above 1.0 in all the cases. So oxygen production on account of algal-photosynthesis is more than sufficient to meet the BOD_5 requirements. The values, decrease as the detention period increases and is always above 1.0 indicating that the oxygenation is due almost entirely to algal photosynthesis.

(vii) Different Phases of bacterial Oxidation in high-rate aerobic Oxidation Pond.

(a) Definition: of Endogenous Metabolism

"Endogenous metabolism can be defined as the sum total of all chemical activities performed by organisms in the absence of utilizable extra-cellular materials serving as sources of energy and building stones for assimilation and growth, water, molecular oxygen and non-carbonaceous and non-nitrogenous mineral salts may be present in external environment and precipitate in endogenous metabolism. In nutritional terms, endogenous metabolism can be viewed as encompassing all these chemical activities engaged in the starving cell".

"Depriving organisms of nutrients causes them immediately to be dependent exclusively upon material

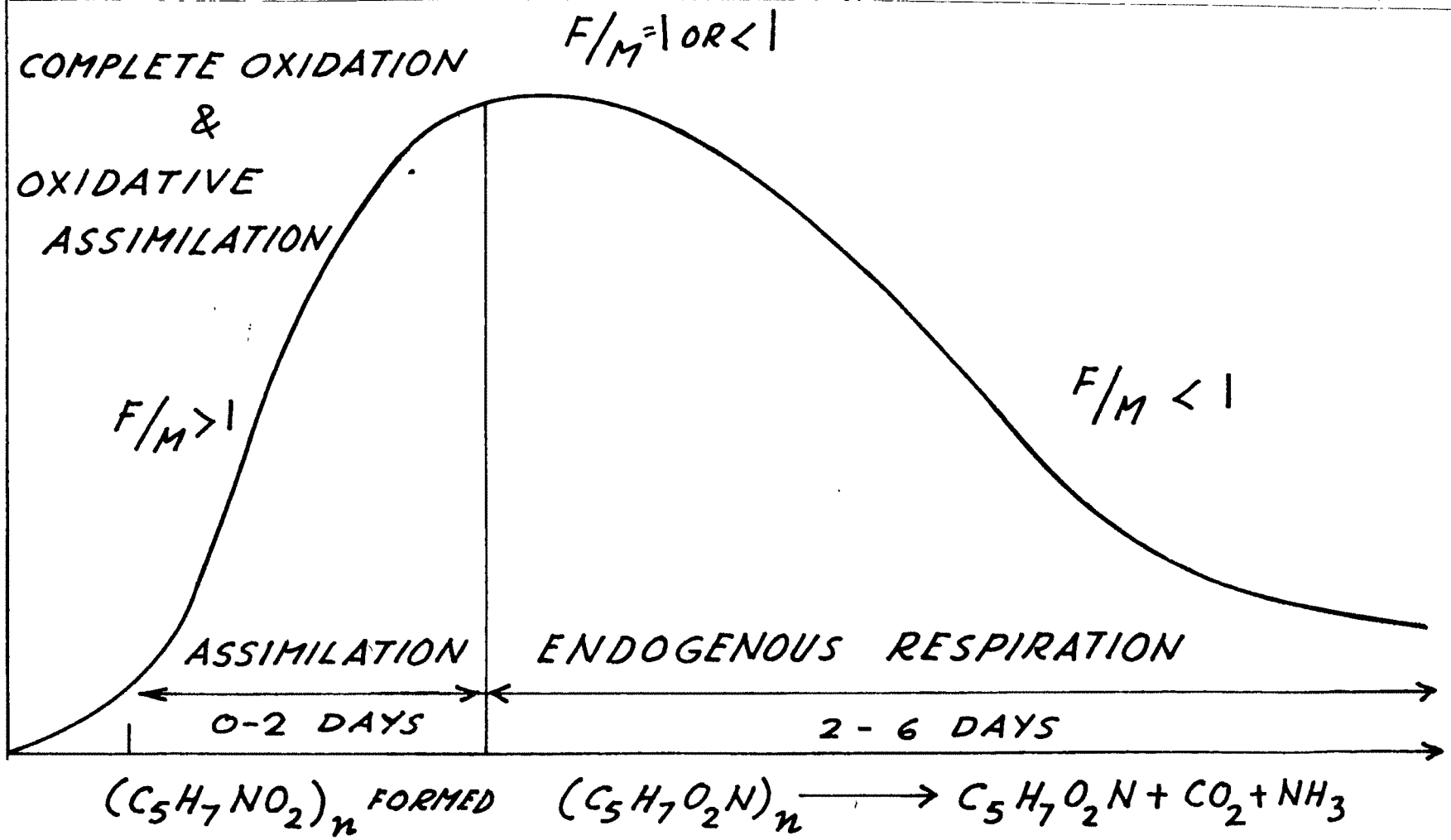
resources for maintaining their lives. At the moment of deprivation of nutrients the cell finds itself with an acquired compliment of enzymes that can continue to act as long as no barriers are interposed to access to endogenous substrates. Thus the metabolic activity of the starving cell could be the expression of an unreasonable but natural compulsion of an enzyme to work with no necessary production end in sight. In fact, a starving cell is losing its substance and must eventually waste away and die. It would be much more efficient if the cell deprived of external resources could automatically stop its endogenous metabolism and rest in a state of suspended animation". (Lamana Carl, 1963)

(b) Different phases of microbial metabolism: (Fig. 8).

Biochemical principles of microbial metabolism of organic matter in biological oxidation processes, represent the kinetics of bacterial biosynthesis and growth and decay; and they are shown below in the four growth phases: In the ideal curve Fig.. the lag phase is largely eliminated in the high-rate

FIG. 8

DIFFERENT PHASES OF BACTERIAL $_{N}$ OXIDATION IN HIGH-RATE AEROBIC OXIDATION POND



Oxidation pond as the pond is inoculated with a large amount of algal-biomsss for starting the photosynthetic process.

The log growth phase may be defined as that period during which regular and maximum multiplication of bacterial sludge takes place. This maximum or logarithemic growth rate is dependent on the mean-generation time of the ecosystem. The generation time is defined as that interval during which one bacterium develops and completely divides into two cells. This results in geometric progression of bacterial growth. As the available food supply is exhausted, a negative accelarative phase exists when cellular division occurs at less intervals.

A stationary phase will follow in which the rate of growth equals the rate of cell death and distruction. When the rate of destruction exceeds the rate of growth, a death phase exists. This is probably the endogenous respiration phase of activated sludge activity. In this phase, the sludge is oxidised to CO_2, water and ammonia.

(c) Application to the Results:

In our experimental studies of three algae i.e. Oscillatoria, spp., Anacystis nidulans , and Scenedesmus

obliqus, they showed that all the inorganic and organic chemical, biochemical tests had high values during the detention period of 0 to 2 days. The nutrients, during these two days were highly used up by bacteria. When the detention period increased from 2 to 4 and from 4 to 6 days that there were gradual decrease of nutrients. It is obvious to conclude that 0 to 2 days detention will represent the assimilatory phase, while 2 to 4 and 4 to 6 days the endogenous phase.

These results are shown in the sub-joined Table - .

P.T.O.

Removal of nutrients during Assimilatory and endogenous phases of Algal-bacterial symbiosis using three algae and Baroda Raw Sewage:

Tests	Detention Period in days. R.S. + Oscillatoria Spp.			R.S. + Anacystis nidulans			R.S. + Scenedesmus obliqus		
	Assimilatory	Endogenesis		Assimilatory	Endogenesis		Assimilatory	Endogenesis	
	0-2	2-4	4-6	0-2	2-4	4-6	0-2	2-4	4-6
A. Organic Nutrients:									
COD	256	60	20	182	42	34	264	80	18
Organic matter	213.3	50	16.6	160	35	28.3	220	66.6	15
Algae formed	288	14	8	192	8	4	248	33	5
B. Inorganic Nutrients:									
Am-N	32.5	2.5	1.2	16.9	4.9	1.3	22.3	1.3	2.4
PO_4	9.4	0.8	1.2	11.5	0.8	0.7	9	0.6	0.9
C. Biochemical Nutrients:									
Carbohydrates									
Free sugar	-	-	-	-	-	-	12.2	14.8	9.2
Total sugar	-	-	-	-	-	-	31	36.1	32.5
Protein	-	-	-	-	-	-	2.2	0.6	1.2
Amino nitrogen	-	-	-	-	-	-	2.3	0.4	0.5
Volatile acids	-	-	-	-	-	-	39.2	29.8	5.0

(B) Algal Photosynthesis:

1. Theoretical considerations:

Photosynthesis is the biological activity where synthetisation of living substance takes place from carbon dioxide water N,P, and other inorganic substances. Radient energy is adsorbed by algae which release free molecular of oxygen as long as light is available. Photosynthetic oxygenation is mainly responsible for the overall stabilization in oxidation ponds. Algae may release 20 times as much oxygen as it is utilized in metabolism (Palmer 1956). Owing to oxidation of organic matter, heterotrophic bacteria produce CO_2 and also liberate ammonia. Green algae utilizing energy from the Sun produce carbohydrates from CO_2,NH_3 and water and assimilate the same together with other products of biological significance for synthesising fresh algal cells each of which is capable of fixing solar energy.

(a) Concepts of algal Growth:

Jewell and McCarty (1968) have summarised the photosynthetic process of algal development as detailed below:

$$A CO_2 + C.NO_3 + ePO_4^{-b} + (C + 3e) + H^+ + \tfrac{1}{2}(b - c - 3e)\, H_2O + \text{Sunlight} \rightarrow CaHbNcOd + Pe + (a + b/4 + 5c/4 - d/2 + Se/4)\, O_2$$

In other words the rate of N (or p) assimilation by algal cells in a function of the rate at which organic material is synthesised.

In most waste treatment systems utilizing algae, algal nutrients flow continuously through the system and thus are constantly renewed.

The theoretical principles of cellular growth in continuous culture was first enunciated by Monod (1949) and its application to algal system was revised by Restosvky (in Malek and Fend, 1966) and Shelef et al. (1968). The importance of this type of process to algal culture is that by manipulation of environmental characteristics (nutrient concentration, light etc.), the algal population can be maintained at steady state or constant population density.

(b) Light Intensity:

Light penetration is directly affected by incident light and inversely affected by depth and culture density. Optimum light intensities for maximum algal growth range from 200 to 400 foot Candles (ft-c) and lower limit may be 100 ft C. Allen (1955) found the growth of Chlorella in sewage was not affected by a

reduction of the daily period of illumination until the extreme of only 4 hours daily was reached, and similarly that a reduction in light intensity from 400 to 20 foot-candles has little effect. So light will not be a limiting factor. Oswald (1963) reported that in laboratory studies with settled sewage, an average of 4% of the incident light energy was fixed by the algal cultures. Conversion efficiency varied inversely with intensity, duration of light and detention time and directly with temperature, and CO_2 concentration. Another possible method of increasing the availability of incident light to individual cells is to remove the algae into the light path by induced mixing as in the high-rate aerobic ponds.

(c) Efficiency of light energy conversion:

In both small and large scale cultures of Chlorella the efficiency is reported to be 12 to 20%, provided the intensity of illumination is not too high (Wassink et al. 1953), Gloyna (1971) has stated that the usual efficiencies range from 2% to 9% with 5% common figures. Ganapati and Srinivasan (1970) have reported a maximum efficiency of 10% in a sewage polluted fish ponds in Madras. Pasche (1960) has reported

7% and Talling (1961) 6%. Myers (1955) has stated that 20% is taken as a reasonable maximum value of efficiency for use of white light. Oswald and Gotaas (1957) found that the overall efficiency under a wide variety of environmental conditions seldom exceeded 10 to 12% of the available energy. Oswald et al. (1957) found the efficiencies of outdoor ponds to range between 1 and 10% with most values in the narrow range of 3 to 7%. Efficiencies of 5% to 8% may be attainable with algal cell concentrations dense to utilize the nutrients and permit harvesting of cells (Gotaas and Oswald 1955). Rabinovistech (1955) has stated that Miss Meffert obtained an average of 8% utilization of total incident light for several months in algal ponds studied by her. Ganapati (1971, p. 330)- has reported a value of 6.6 to 13.8 in Indian oxidation ponds.

(d) Temperature:

As in case of all organisms temperature affects the growth rate of algae normally following the Vant Hoff rule according to which growth rate doubles for each 10°C in rise in temperature, within the range of temperature tolerance.

van 't Hoff

(e) Carbon Source:

It is usually the limiting elements when algae are cultured in sewage. Algae normally use free CO_2 as an inorganic C source, though some algae are reported to use the bicarbonate ion also. Use of artificially introduced CO_2 is neither essential nor desirable when algae are cultured in sewage for photosynthetic oxygen production, because the culture may obtain the same from air. It is established that air contains 0.03% CO_2 normally; and this amount is adequate to sustain maximum photosynthetic efficiency if a sufficient volume of the mixture is brought into contact with cell surface (Davis, 1953). The 1-5% CO_2 in air mixture seems to be needlessly large. Any device which provides for more even dispersal of the gas will make possibly a lower CO_2 percentage or a lower total gases flow. The major portion of the gaseous CO_2 bubbled through most cultures is lost. So, the practice of swerling or bubbling air through as a result of mixing replenishes CO_2 by increasing the surface area exposed to the atmosphere.

The maintenance of a reservoir of energy and nutrients for a long enough time of 2-6 days so as to permit an economically large harvest of algae is

effected by diluting the growing population with fresh medium at a rate commensurate with the rate of growth. The slow continuous flow of fresh medium into the culture and the out-flow of medium and cells hold the mutual self-shading. Active photosynthesis causes the pH to increase to 10 or more according to the absorption of atmospheric CO_2 by the culture. Under such conditions, this CO_2 appears as a bicarbonate ion and becomes available to algae immediately. So, algae may compensate for a shortage of CO_2 by increasing the CO_2 absorbing properties of the liquid in which they grow, i.e. by becoming more alkaline or by increase in pH. When all the available free CO_2 is used up the half-bound CO_2 (bicarbonates) from 4/5 to 5/8 is then availed and used up (Birge and Juday 1911). When the first two sources are exhausted, the fully bound CO_3 (or mono-carbonates) may also have to be used up (Schutow, 1926; Maucha, 1929; Neresheimer and Ruttner, 1929; Juday, Birge and Melocke, 1935).

(f) Inorganic nutrients:

Ammonia-nitrogen, nitrous nitrogen and nitric nitrogen and orthophosphate are included under the head. Most algae are able to utilize either Am-N or

nitrate-nitrogen and also nitrite nitrogen if the concentration is very low (about 0.001 molar) according to Fogg and Wolfe (1954). But they prefer Am-N to NO_3-N when both sources are provided in the same culture (Harvey, 1940; Schular et al. 1953).

The use of either of these N sources can cause undesirable changes in pH according to Cramer and Meyers (1948) as detailed below:

$$1.0(NO_3) + 5.7(CO_2) + 5.4\ (H_2O) \longrightarrow C_{5.7}H_{9.8}O_{2.3}N_{1.0}\ 8.25\ O_2 + 1.0\ (OH^-)$$

$$1.0(NH_4^+) + 5.7\ (CO_2) + 3.4\ (H_2O) \longrightarrow C_{5.7}H_{7.8}O_{2.3}N_{1.0} + 6.25\ O_2 + 1.0\ (H^+)$$

Thus nitrate assimilation results in the production of OH^- ions which causes a rise in pH; whereas Am assimilation lowers the pH by formation of H ions.

Many blue-green algae can fix atmospheric N (Fogg, 1947) when an extremely low concentration of dissolved N is available in the medium. So, for the nitrogen removal nitrogen fixing algae should not be used in a treatment plant.

Algae use P as orthophosphate (PO_4^{-3}). The ratio of N:P in a typical algal cell is about 8 - 10:1 and

is essential to algal growth and without it no growth will take place. Sawyer (1952) found that N:P ratios in natural waters where algal blooms prevailed varied from 30 : 1 to 15 : 1 depending upon the species of algae. Some algae can "Store" P ion the element upto a certain concentration when provided with quantities in excess of their requirements (Ketchum, 1939). Zabat et al. (1970) found that maximum uptake was 2% of the cellular dry weight; P uptake can be influenced by light (Gest and Kamen, 1948) and H ion concentration effects the availability of P.

Micro-nutrients are always present in sewage.

Myers (1962) has stated that 1.8 mg of carbon dioxide are used up during the formation of 1.0 mg of dry algal matter; and Oswald and Gotaas (1957) have found that 1.6 mg of oxygen are released during the formation of 1.0 mg of dry algae. On the basis of the above two assumptions, the following calculations are made.

2. Application to connected results:

(i) Calculation of CO_2 used up during Photosynthesis (mg/l).

Detention period in days	Oscillatoria Spp. Alga formed	Oscillatoria Spp. CO_2 used up	Anacystis nidulans Alga formed	Anacystis nidulans CO_2 used up
2	288	518.4	192	345.6
4	302	543.6	200	360.0
6	310	558.0	204	367.2

(ii) Quantity of photosynthetic O_2 released into the biosystem(mg/l)

Detention period in days	Oscillatoria Spp. Alga formed	Oscillatoria Spp. O_2 produced	Anacystis nidulans Alga formed	Anacystis nidulans O_2 produced
2	288	460.8	192	307.2
4	302	483.2	200	320.0
6	310	496.0	204	326.4

3. Overall Photosynthetic Energy Conversion efficiency:

1. Theoretical considerations:

Gotaas and Oswald (1955) and Oswald and Gotaas (1957) have developed an input and output energy balance system for estimating overall photosynthesis energy conversion efficiency, in which a basic assumption is made that the system under study is a continuously stirred reactor (STR) with complete homogenity of the algal cells in suspension. (Beck et al. (1969). As in any continously stirred tank reactor there is a finite volume V in litres and flow rate F, in litres per day. The mean hydraulic residence time ϕ is then defined as

$$\phi = V/F \text{ ---------- (1)}$$

For a given mean residence time ϕ, in days, the total solar energy input per litre of pond volume is equal to

$$E_{in} = S.A.\ \phi \text{ ---------- (2)}$$

Where E_{in} is the total energy input in calories per litre; S, the daily solar energy input in calories per square cm per day; A, the surface area of one litre

of pond volume receiving Sunlight in Cm^2 per day and ϕ is the mean hydraulic residence time. For one litre of pond volume A = 1000/d where d is the determined depth in cm.

$$\text{Therefore } E_{in} = \frac{1000\ S.\ \phi}{d} \qquad (3)$$

The energy output in the form of synthesised algae is defined as

$$E_{out} = h.\ C_c \qquad (4)$$

Where E_{out} is the total energy tied in synthesised algae in calories per liter, h is the heat of combustion of algae in calories per milligram, and C_c, is the concentration of algae in mg/l. Equations (2) and (4) can be equated by assuming that only a portion of the energy input is converted to algal biomass, so that:

$$E_{in}e = E_{out} \text{ ----------} \qquad (5)$$

Where e is an efficiency factor, the equation (5) can be expanded thus

$$\frac{1000.\ S.\ \phi.1}{d} = h.\ C_c \quad (6)$$

$$\text{and } \therefore \quad e = \frac{h.\ C_c.\ d}{1000 - S.e} \text{ ----------} (7)$$

Myers (1964) recommends a value of 5.5 calories per mg as the heat of combustion h of algae.

(ii) Application to connected Results:

The above equation is used in calculating the overall photosynthetic energy conversion of two algae i.e. Oscillatoria Spp. and Anacystis nidulans. The pertinent data used in our calculations are shown for the algae Oscillatoria spp. and Anacystis nidulans used in both of our laboratory experiments respectively.

(a) Oscillatoria spp.

Volume of the culture fluid i.e. raw sewage used = 1.5 litre. Depth of the liquid volume = 3.5 cm. Maximum quantity of algal biomass produced in 6 days in 1.5 litres sewage = 310 + 155 = 465 mg.

Average light energy available in the lighted room = 375 lux.

10.5 lux = 1 gram - calorie

∴ 1 mg of algae contains 5.5 calories of heat of combustion.

Applying these values in the equation (7) we get

$$e = 465 \times 5.5 \times 3.5 / (35.7 \times 1500 \times 6)$$

$$= 0.0280$$

$$\text{or} = 0.0280 \times 100$$

$$\text{or} = 2.80\%$$

(b) Anacystis nidulans

Volume of the culture fluid i.e. raw sewage used = 1.5 litres

Depth of the liquid = 3.5 cm.

Maximum quantity of algal biomass produced in 6 days in 1.5 litres of sewage = 204 + 102 = 306 mg.

Average light energy available in lighted room = 375 lux.

10.5 lux = 1 gram - calorie

∴ 375 lux = 35.7 gram - calorie

1 mg of algae contains 5.5 calories of heat of combustion.

Applying these values in the equation we get:

$$e = 306 \times 5.5 \times 3.5/35.7 \times 1500 \times 6$$
$$= 0.0183$$

or = 0.0183 x 100

or = 1.83%

The Calculated results of photosynthetic efficiency of the respective algae are given in a tabular form below:

Name of Algae	% Photosynthetic efficiency
1) Oscillatoria spp.	2.80
2) Anacystis nidulans	1.83

(C) Bio-stimulatory nutrients and their removal during photosynthesis:

1. Theoretical Consideration:

Two groups of nutrients are involved in algal-bacterial symbiosis i.e. major and micro-nutrients. The former consists of C, N and P and the latter of trace elements like Cu, Mn, Sb, As etc. The major nutrients are used up by heterotrophic bacteria and autotrophic algae, the two main partners in the game of algal-bacterial symbiosis.

Stumm (1968) has laboured to show that not only the main constitutional elements in both bacterial and algae are the same (C,N and P) but are also found in the same constant proportions of 106 : 16 : 1 atoms. Therefore, in the high-rate aerobic oxidation pond methods of waste treatment the ratios of C:N and C:P are very important in view of their utilization in algal and bacterial symbiosis.

Public Health Engineers usually express the carbon value in terms of BOD_5. The biological oxygen demand is a measure of the carbon oxidized where one molecule of carbon combines with one molecule of oxygen.

Then the carbon to nitrogen that is available to both the types of organisms are considered and it may be present as Am ion from salts, urea or products of hydrolysis (Porges, 1960). Beside, literature shows that nitrogen enters synthesis reactions of bacteria or algae at the oxidation level of ammonia. Therefore, if any other N source except Am is to be utilized, it must first be converted into Am before it becomes available. There are well defined over-all reactions which depict the conversion of NO_3-N and NO_2-N to Am. These other two forms of N are therefore, available for use in production of bacterial protoplasm (Symons and Mckinney 1958, p. 875).

2. Application to the connected Results:

(i) Carbon consideration:

Organic carbon is mainly utilized in algal-bacterial symbiosis. It is a difficult task to compare oxygen output with carbon dioxide uptake resulting from bacterial oxidation of organic matter in algal-bacterial symbiosis in high-rate aerobic pond systems.

So, photosynthetic oxygen values are used for calculating the organic carbon used up according to

Vollenweider (1969 p.117): "Assuming that the gas exchanges are the results of photosynthetic production of the conventional hexose, the photosynthetic quotient (P, Q=O_2 output/CO_2 uptake by volume), should be unity and hence: g.O_2 x 0.375 = g.carbon".

Since the oxygen demand is a measure of carbon oxidized, and one molecule of carbon combines with one molecule of oxygen the ratio of 12 : 16. So carbon values have been estimated in two ways (a) from photosynthetic O_2 production and (b) from the COD values; the values for organic matter are calculated according to Porges (1960, p.23) and the two values are compared below:

Detention period in days	Organic matter used up from COD values(Porges) (mg/l)	Organic carbon used up from photosynthetic O_2 (mg/l)
Oscillatoria spp.		
2 days	213.3	171
4 days	263.3	180
6 days	280.0	186
Anacystis nidulans		
2 days	152	114
4 days	187	120
6 days	215	122

~~The values for organic carbon and organic matter from COD values are not the same. The N values calculated from photosynthetic oxygen are less.~~

Various studies on waste water purification show the optimum rates of carbon to nitrogen used up in stabilization. In terms of the ultimate oxygen demand or COD, the COD to nitrogen ratio is found to be 25:1 (Porges, 1960). Sawyer (1956) has reported a wide BOD to N ratio of 32:1, equal to C to N ratio of 17.5 to 1 with longer periods of detention.

In our own case the following ratios have been found:

Stabilization ratio of carbon to nitrogen as	Ratio obtained by using *Oscillatoria* spp.		*Anacystis nidulans*	
	Actual	Reduced	Actual	Reduced
BOD_5 to N	260:36.2	7.18:1	152:23.1	6.58:1
COD to N	336:36.2	9.28:1	258:23.1	11.2 :1
or				
C : N	252:36.2	6.96:1	195:23.1	8.44:1
Org. C to N	186:36.2	5.14:1	122:23.1	5.28:1
Org. matter to N	280:36.2	9.73:1	215:23.1	9.31:1

The stabilization ratios are found to be lower in algal-bacterial symbiosis taking place in high-rate aerobic ponds.

(ii) Ratios of COD and Organic carbon to Phosphate-phosphorus:

It has been stated that the critical levels are much lower than N with the BOD_5 to P varying from 9 to 1 upto 150 to 1, indicating that one unit of P is required for 49 to 82 units of C (Porges, 1960).

In the case of our two algal growth cultures the following ratios have been found in respect of BOD_5 to P, COD to P and organic carbon to P during algal-bacterial symbiosis.

Stabilization ratio of carbon to phosphorus as	Ratio obtained by using			
	Oscillatoria spp.		Anacystis nidulans	
	Actual	Reduced	Actual	Reduced
BOD_5 to P	260:2	130:1	152:4.5	33.8:1
COD to P	336:2	168:1	258:4.5	57.3:1
or C to P	252:2	126:1	195:4.5	43.3:1
Organic C to P	186:2	93:1	122:4.5	27.1:1
Org. matter to : P	280:2	140:1	215:4.5	47.8:1

The case of Oscillatoria spp. one unit of P is required 93 to 168 units of carbon in this particular case, while in case of Anacystis nidulans rates are

are found to be lower in algal bacterial symbiosis taking place in high-rate aerobic pond. The uptake of P is influenced by light (Gest and Kaman 1948) and its availability is influenced by the pH of the medium.

(iii) Ratio of N:P in algal Photosynthesis:

Sawyer (1971) has recently recorded that the primary producers use nitrogen and phosphorus in a ratio of 15:1 and that natural bicarbonate activity serves as an adequate source of carbon dioxide for algal blooms in all wastes excepting acid.

The N:P ratio in the Algae studied has been calculated below:

Algae	The Ratio of N (Utilized) Total Utilization		P:Utilized in 6 days Algal Synthesis Utilization	
	Actual	Reduced	Actual	Reduced
Oscillatoria spp.	36.2: 3.8	9.53:1	25.11: 3.2	7.8:1
Anacystis nidulans	23.1: 4.5	5.13.:1	15.99: 2.20	7.27:1

(iv) Nitrogen Utilization in Algal Photosynthesis:

Nitrogen assimilation is calculated on the basis of the amount of Am-N used up from the ecosystem as a result of algal growth. This is an over-simplification of the real system found in the growth cultures where decomposition of organic matter, bacterial cellular synthesis and

endogenous respiration and algal growth are taking place almost simultaneously and a constant flux of nitrogen forms take place. The Am-N nitrogen consumed in the two algal growth cultures are shown below:

<u>Oscillatoria</u> spp. (mg/l)

Detention time (days)	Algal growth in (mg/l)	Am-N (mg/l) Found	Am-N (mg/l) Used %	N-content of algal biomass (mg/l) 8.1%	%Utilization of Am-N by algal biomass
0	-	37.8	-	-	-
2	288	5.3	32.5(85.98)	23.33	61.72
4	302	2.8	35.0(92.69)	24.46	66.71
6	310	1.6	36.2(95.77)	25.11	66.42
Final % reduction			95.8		

<u>Anacystis nidulans</u> (mg/l)

Detention time (days)	Algal growth in (mg/l)	Am-N (mg/l) Found	Am-N (mg/l) Used %	N-content algal biomass (mg/l) 7.84%	%Utilization of Am-N by algal bio mass
0	-	28.8	-	-	-
2	192	11.9	16.9(58.68)	15.05	52.25
4	200	7.0	21.8(75.69)	15.68	54.44
6	204	5.7	23.1(80.20)	15.99	55.52
Final % reduction			80.2		

= =

80.2 to 95.8% of the Am-N has been utilized in 6 days during the treatment processes in the Anacystis nidulans and Oscillatoria spp. growth units. The nitrogen content of algal cells was estimated and found in the 7.84% in Anacystis and 8.1% in Oscillatoria so that the N content of 15.99 mg/l and Anacystis nidulans and of Oscillatoria spp. 25.11 mg/l. So the utilization of Am-N by the Anacystis nidulans is 55.52% while in the case of Oscillatoria is 66.42%. So 25 to 30% of the Am-N must have been utilized for other biochemical reactions.

Algae require N either as Am-N or NO_3-N but they seem to prefer Am-N when both are provided together (Harvey 1940; Schuler et al. 1953). It is also reported that nitrate assimilation results in the production of (OH) ions which caused a rise in pH while Am-N assimilation lowers the pH on account of the formation of H ions.

(v) Phosphorus Consideration:

Phosphorus is used as orthophosphate in algal bacterial symbiosis. Assuming that it is used in algal growth reactions the following calculations are made. The amount of phosphorus as P and PO_4 used during the algal

bacterial symbiosis. 75 to 97% of the phosphate-phosphorus has been used in the process of algal-bacterial symbiosis and 36 to 77% has been used by the two alga. The wide difference in quantity is perhaps due to the different types of algae studied and also to precipitation by phosphates of Ca and Mg due to high pH values reached in six days detention period.

Oscillatoria spp. (mg/l)

Detention period in days	Algal growth (mg/l)	PO_4-P in Eco-system Found		Used		% Reduction		P-content of algal biomass 1.04%	%Utilisation of P by algal biomass
		PO_4	P	PO_4	P	PO_4	P		
0	-	12.5	4.2	-	-	-	-	-	-
2	288	2.9	0.9	9.6	3.3	76.96		2.99	71.19
4	302	2.3	0.8	10.2	3.4	81.61		3.14	74.96
6	310	1.1	0.4	11.4	3.8	91.2		3.22	76.66
Final % reduction						91.2			

* In the ecosystems the following reactions are taking place simultaneously (i) Decomposition of organic matter (ii) bacterial cellular synthesis (iii) endogenous respiration and (iv) algal growth.

The organic matter from ecosystem is removed as bacterial growth, while nutrients are moved as algal growth.

Anacystis nidulans (mg/l)

Detention period in days	Algal growth (mg/l)	PO_4-P in Ecosystem Found		Used		% Reduction	P-content of algal biomass 1.08%	%Utilization of P by algal biomass
		PO_4	P	PO_4	P			
0	-	18.3	6.1	-	-	-	-	-
2	192	6.8	2.3	11.5	3.8	62.84	2.07	33.93
4	200	6.0	2.0	12.3	4.1	67.21	2.16	35.41
6	204	4.7	1.6	13.6	4.5	74.86	2.20	36.06
Final % reduction						74.86		

(a) Nutrients in raw settled sewage:

The major nutrients are C, N and P and micronutrients are always present. The porportions in which the major nutrients present in our sewage samples are shown under:

Nutrient ratio	Raw Sewage Oscillatoria spp. Actual	Reduced	Anacystis nidulans Actual	Reduced
C. N	280:37.8	7.4:1	166:28.8	5.76:1
C. P	280:12.5	22.4:1	166:18.3	9:1
C.COD BOD_5	12,372:280	12.31:23.3	12,280:166	12,23.3:13.8
N. P	37.8:12.5	3.02:1	28,8:18.3	1052:1

Porges (1960) has found the C : COD :BOD_5-ratio as 12 : 32 : 21.9. Our corresponding values are lower.

Sawyer (1971) has recently recorded the N:P ratios of waste waters. He has stated that the predetergent domestic sewage was slightly rich in phosphorus with a N:P ratio of 10 and that the modern domestic sewage has N:P ratio of 3, and is excessively rich in phosphorus. In our case we get the N:P ratio as 3.02 raw sewage used for Oscillatoria spp. and 1.5:1 in raw sewage used for Anacystis nidulans:

Carbon is deficient in domestic sewage for utilizing all nitrogen and phosphorus into bacterial solids in the above proportions.

(b) Nutrients in algal-bacterial symbiosis:

Sawyer (1971) has stated: Carbon, nitrogen and phosphorus form the three important constituents of algal tissues, where carbon alone account for 35 to 50 percent. Kuentzel (1969, 1970, 1971) therefore, considers that the complete removal of organic matter from waste water is necessary for preventing eutrophication in receiving water. So, algal growth can never be controlled solely by the removal of phosphorus alone. Nitrogen and phosphorus containing substances are furnished by domestic waste waters.

(D) Algal-bacterial Symbiosis in High-rate Ponds:

1. Theoretical Consideration:

Two broad groups of organisms are mainly involved in the process. They are : (a) heterotrophic organisms consisting of consumers i.e. protozoans and decomposers i.e. bacteria. The role of the latter is far more important than the former and therefore will be considered The autotrophs are the microscopic algae: greens, blue-greens and diatoms.

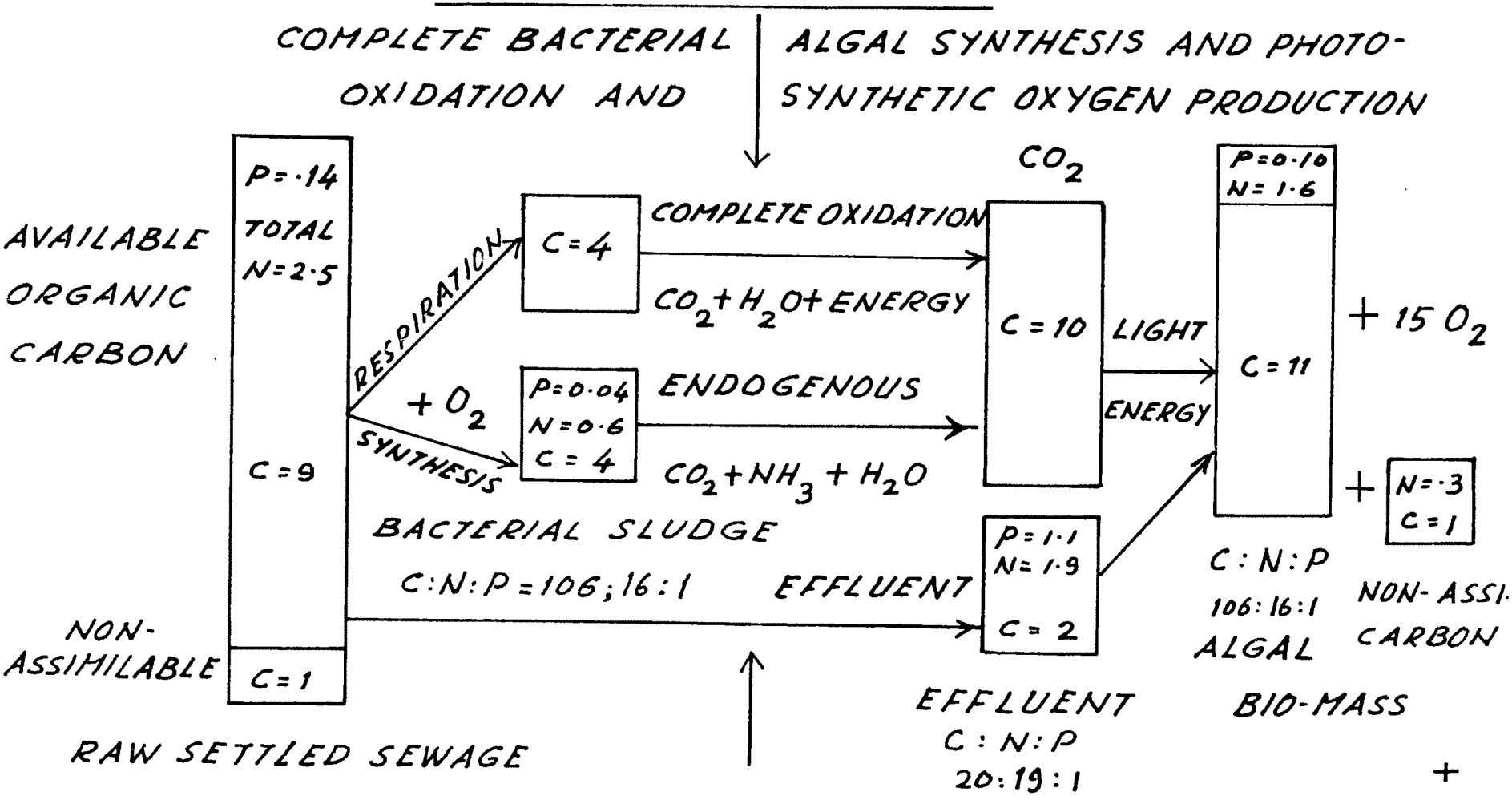
(i) Stoichiometry of Algal-bacterial symbiotic system: (Fig. 9)

The basic principles of the symbiotic system are (a) the production of organic matter (algae) is accompanied by the absorption of radiant energy from the Sun and the concomitant release of nascent oxygen, while the destruction of organic matter involves the utilization of an almost equivalent amount of oxygen and release of energy. The final degradation of products of aerobic bacterial oxidation of organic matter are CO_2, NH_3 and H_2O which are identical to the chief needs of algal photosynthesis plus radiant energy.

Oswald, Hee and Gotaas (1958) found experimentally in high-rate aerobic ponds the oxidation of sewage organic matter to follow the reactions.

FIG. 9

BIOCHEMISTRY OF ALGAL-BACTERIAL SYMBIOSIS IN HIGH RATE AEROBIC POND SYSTEM (MODIFIED AFTER STUMM 1968)



$$C_{11}H_{29}O_7N + 14O_2 + H^+ \longrightarrow 11CO_2 + 13H_2O + NH_4^+$$

Oxidation to nitrate of the ammonia formed rarely occurs because ammonia is assimilated by algae, lost to the air or precipitated during periods of high pH before nitrification is established.

Bacteria present in waste water on the other hand decompose organic matter in a series of redox reactions and the overall Stoichiometry according to Stumm (1968) may be represented by the equation:

$$C_a H_b N_e O_d P_e + (A + \tfrac{1}{4}b - \tfrac{1}{2}d + 3/2c + 2e)O_2 =$$
$$CO_2 + b/2H_2O + c\,NO_3 + ePO_4$$

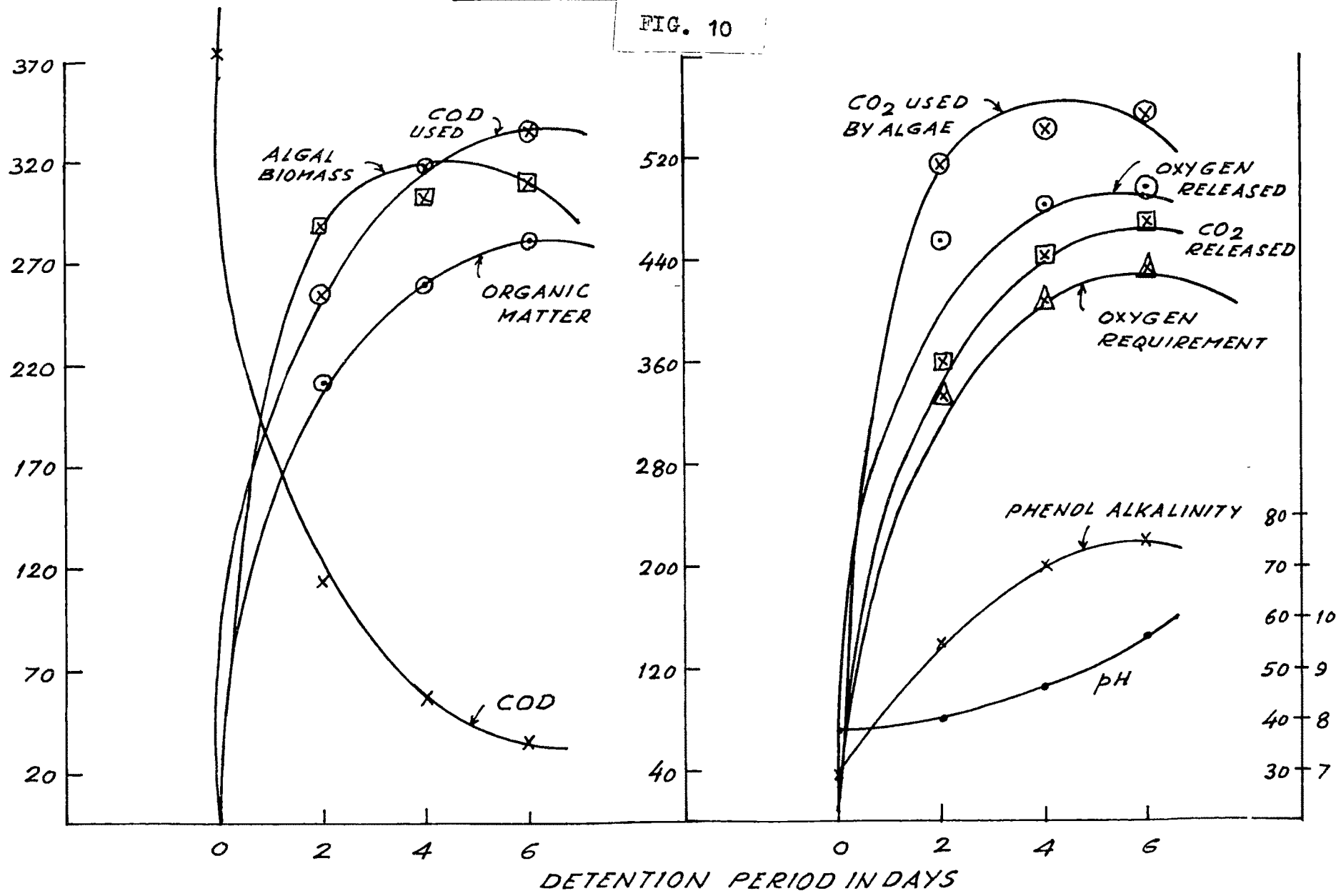
The resultant products are soluble inorganic nutrient substances containing CO_2, NO_3 and PO_4 which are returned to the ecosystem. These nutrients are utilized by algae in the presence of radient energy for further synthesis according to the equation (Stumm 1968)

$$106\,CO_2 + 90H_2O + 16NO_3 + 1PO_4 + \text{radient energy} =$$
$$\underset{\text{Algae}}{C_{106}H_{180}O_{45}N_{16}P} + 154\tfrac{1}{2}\,O_2$$

The Stoichiometric relation between C, N and P in algal mass = 106:16:1 atoms (Stumm, 1968).

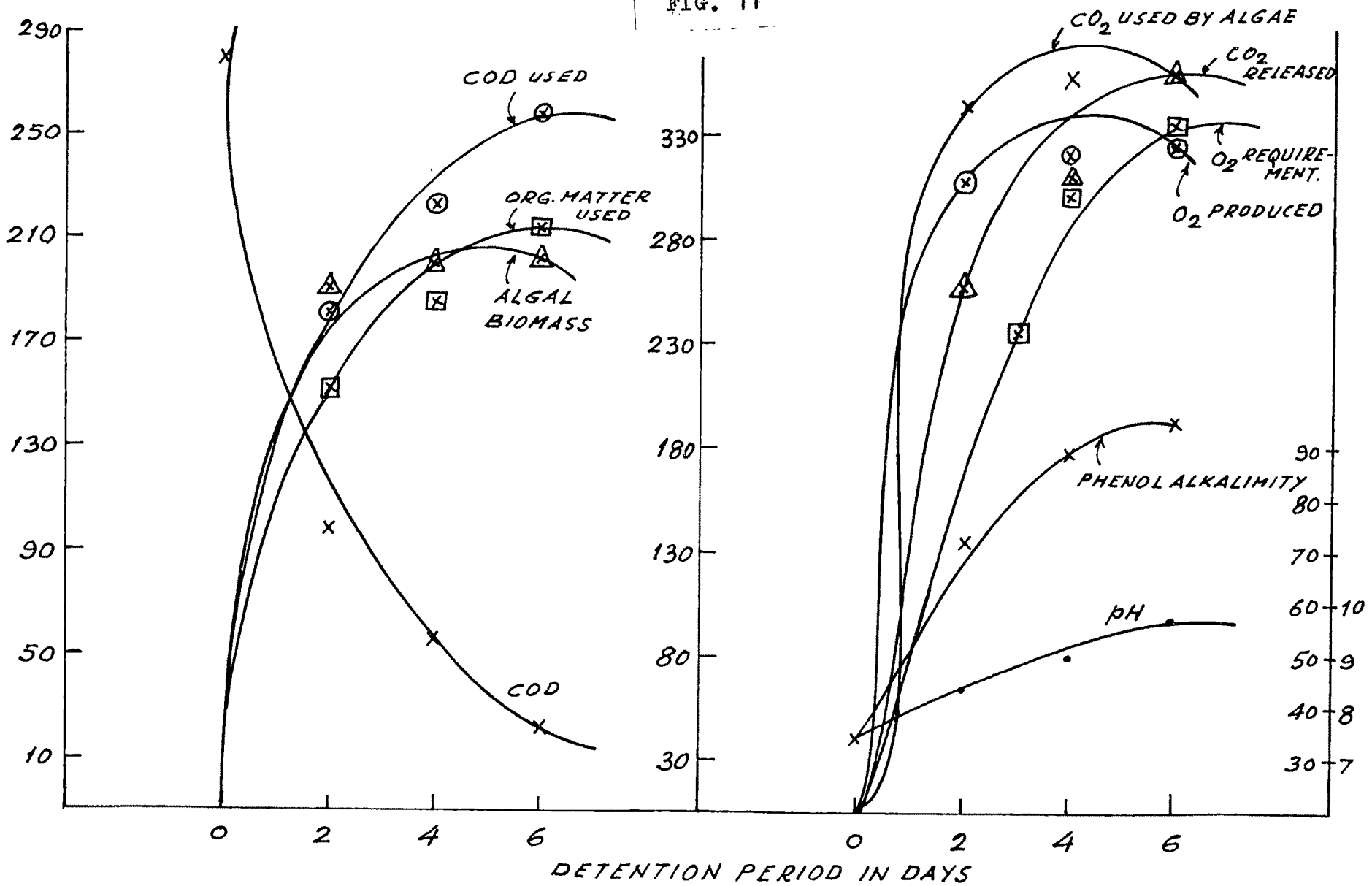
ALGAL-BACTERIAL SYMBIOSIS IN BARODA RAW SEWAGE WITH OSCILLATORIA spp.

FIG. 10



ALGAL-BACTERIAL SYMBIOSIS IN BARODA RAW SEWAGE WITH ANACYSTIS NIDULANS

FIG. 11



2. Application to connected Results: (Fig. 10 & 11)

(i) Quantity of O_2 released during algal photosynthesis is compared with the O_2 required for total bacterial oxidation of polluting organic matter during algal bacterial symbiosis in high-rate oxidation ponds in connection with the two algae experiments:

(i) Oscillatoria Spp. O_2 (expressed in mg/l)

Detention period in days	Released during algal photosynthesis.	Required for bacterial oxidation	Difference (excess %)
2	456.8	332.7	124.1(37.4%)
4	483.2	410.7	72.5(17.6%)
6	496.0	436.8	59.2(13.5%)

Photosynthetic oxygen production is in excess of the quantity of oxygen required for bacterial oxidation of polluting organic matter by about 13% to 37% during algal-bacterial symbiosis. It indicates naturally that the ecosystem is always aerobic

(ii) Anacystis nidulans: O_2 (expressed in mg/l)

Detention period in days	Released during algal photosynthesis	Required for bacterial oxidation	Difference (excess %)
2	302.2	237.1	70.1(29.5%)
4	320.0	291.7	8.3(2.8%)
6	326.4	335.4	-9.0(?)

= =

There is an excess of photosynthetic oxygen over that required for total bacterial oxidation on account of greater algal biomass production so that the ecosystem is kept aerobic. There is a slight deficit in photosynthetic O_2 content towards the end which may be accounted for by atmosphere reaeration. Such cases are very rare.

(ii) CO_2 released during total bio-oxidation of polluting organic matter is compared with the quantity used up for algal biomass production in algal-bacterial symbiosis of the two algae used in our experiments: (Fig. 9 and 10).

Detention period in days	Used in algal photosynthesis	From bacterial oxidation	Difference (excess %)
Oscillatoria spp. (CO_2: mg/l)			
2	518.4	360.5	157.9(43.8%)
4	543.6	445.0	98.6(22.1%)
6	558.0	473.2	84.8(17.92%)
Anacystis nidulans (CO_2: mg/l)			
2	345.6	256.9	88.7(34.5%)
4	360.0	310.0	50.0(16.12%)
6	367.2	363.3	3.9(1.1%)

(iii) Excess of CO_2 released and used in algal Photosynthesis explained:

From a study of the two tables (i and ii) relating to the two algae investigated and shown above, It will be seen that the CO_2 released from bacterial oxidation of sewage organic matter is much less than required for algal photosynthesis during algal-bacterial symbiosis. The deficiency ranges from 1 to 44%, of the quantity released during total bacterial oxidation; and the excess required for the algal biomass formed should have come only from the atmosphere and the bicarbonate-carbonate equilibrium system. The availability of the bicarbonate ion per se for photosynthesis is an important factor.

The importance of the relationship between free carbon dioxide and alkalinity with respect to pH has been described by King (1970), "Atmospheric CO_2 is also used up by algae during algal-bacterial symbiosis (Oswald, 1960).

3. Regression analysis of nutrient strength expressed as COD and algal biomass and correlation coefficients between the two algae: i.e. Oscillatoria spp. and Anacystis nidulans:

The nutrient strength of a waste is ordinarily expressed in terms of the Biochemical Oxygen Demand or BOD, which is a measure of the quantity of oxygen required to carry out the aerobic bio-oxidation of the biologically available organic material in waste under specific conditions of time and temperature (Standard methods, 1971). Oswald and Golucke 1960, p. 229) have found that in steady state continuous cultures under specific conditions of light intensity, temperature and other factors, there is an optimum BOD for algal growth, and that upto this optimum, algal growth increases almost linearly with increased BOD. They have also added that decrease in concentration of algae occurs at BOD concentrations in excess of this optimum, probably because strong waste contains excess colloidal material and bacterial which remains in suspension and thus decrease the energy available for algal growth.

Our studies confirm the observations of Oswald and Golueke (1960). Under our laboratory batch-culture experimental conditions of light intensity, temperature and other factors and using Baroda, settled and strained sewage, we find a high degree of direct correlation between algal growths of the

two blue-green algae and their corresponding used up CODs. Regression analysis relating to algal growth in each case with corresponding used up COD have been worked out for the individual as well as the algae together. The correlation coefficient "r" for each algal is also indicated in the tabular statement. Along with the corresponding values for the two constants "m" and "b" in the regression equation.

The regression equation is as follows:

$$x = my + b$$

where x represents the COD values

y represents the Algal biomass

(i) Regression analysis constant "m"

$$\frac{n.\Sigma xy - \Sigma y.\Sigma x}{n.\Sigma y - (\Sigma y)^2}$$

(ii) Regression analysis constant "b"

$$\frac{y^2\Sigma x - \Sigma y.\Sigma xy}{n.\Sigma y - (\Sigma y)^2}$$

(iii) Correlation coefficient "r" =

$$r = \frac{n.\Sigma xy - \Sigma y - \Sigma x}{(n\Sigma x^2) - (\Sigma x)^2 \quad (n\Sigma y^2 - (\Sigma y)^2}$$

FIG. 12

FREE SUGAR USED AND REMAINING IN THE EXPERIMENT WITH SCENEDESMUS OBLIQUS

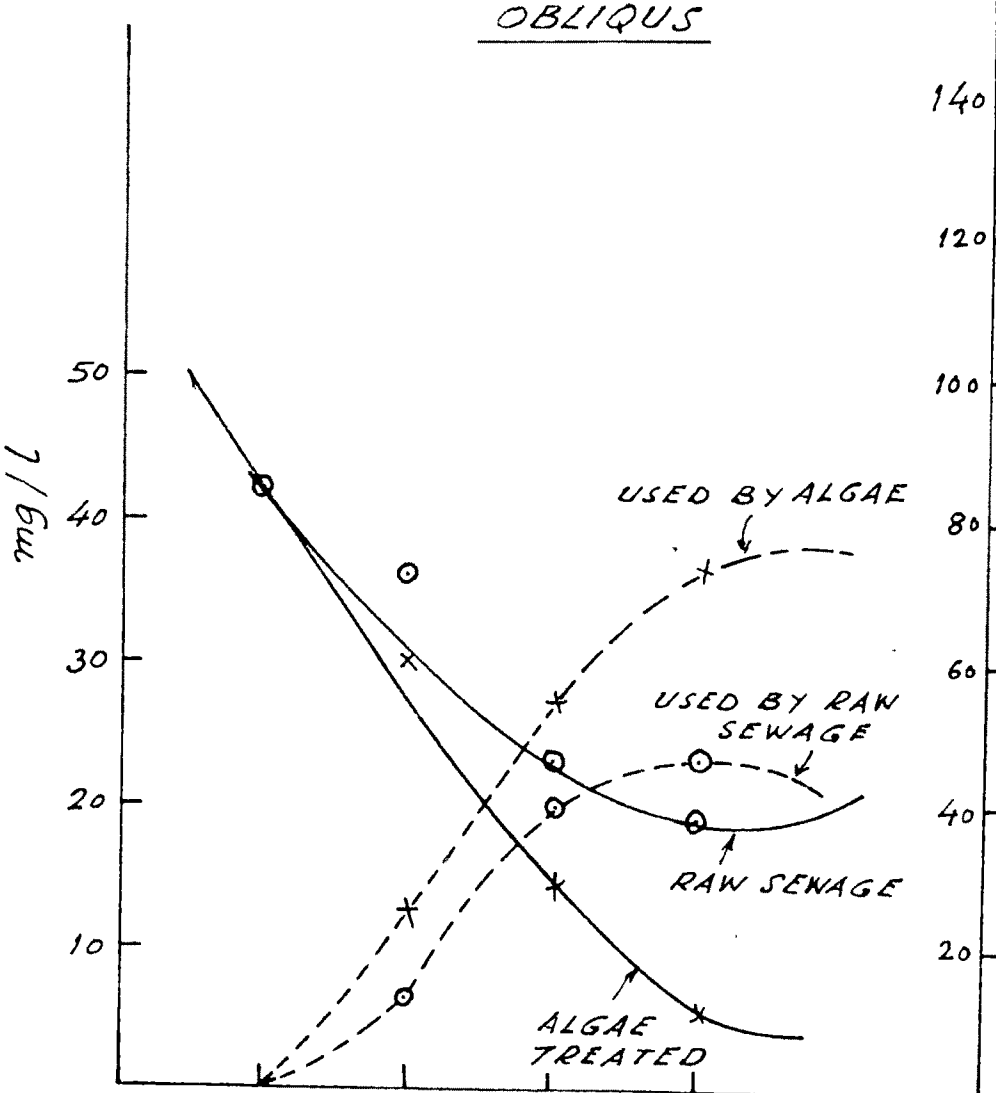
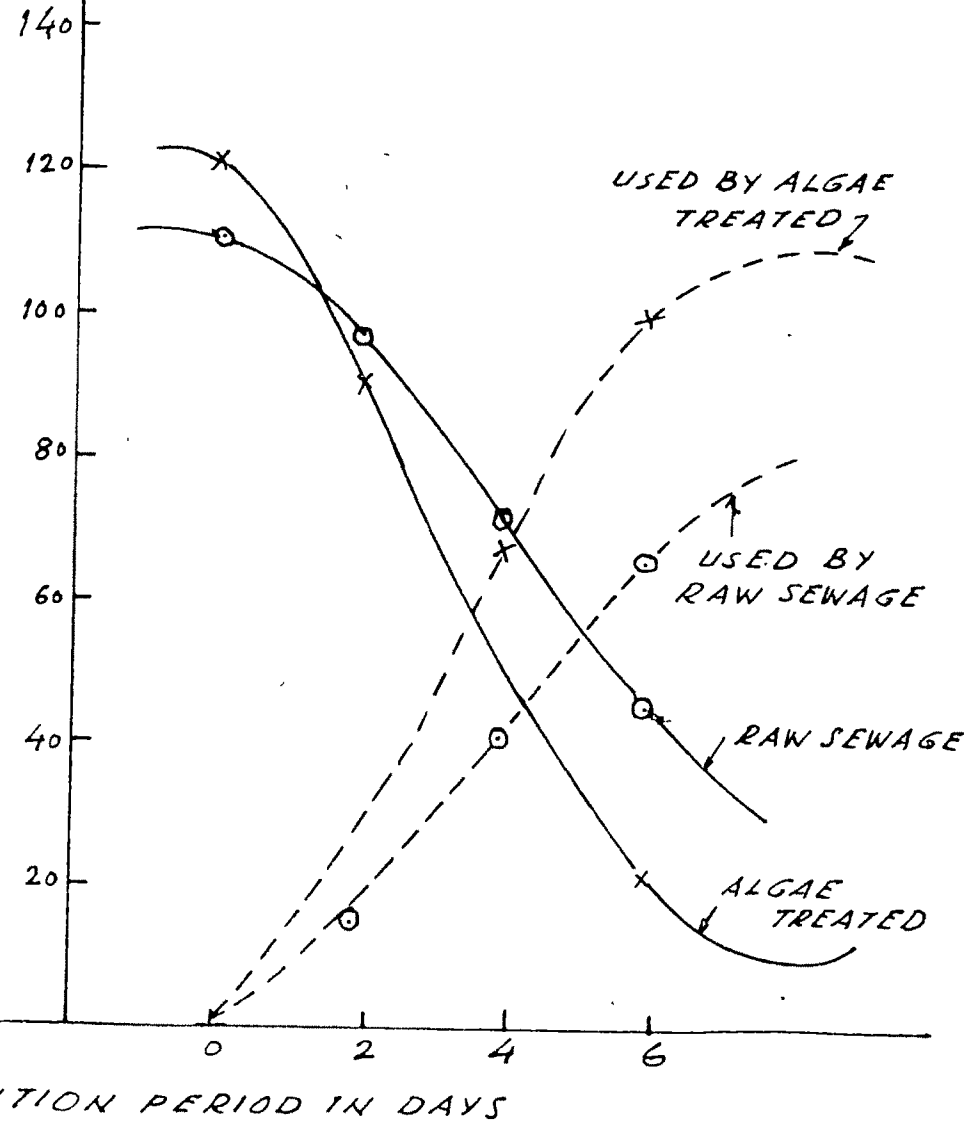


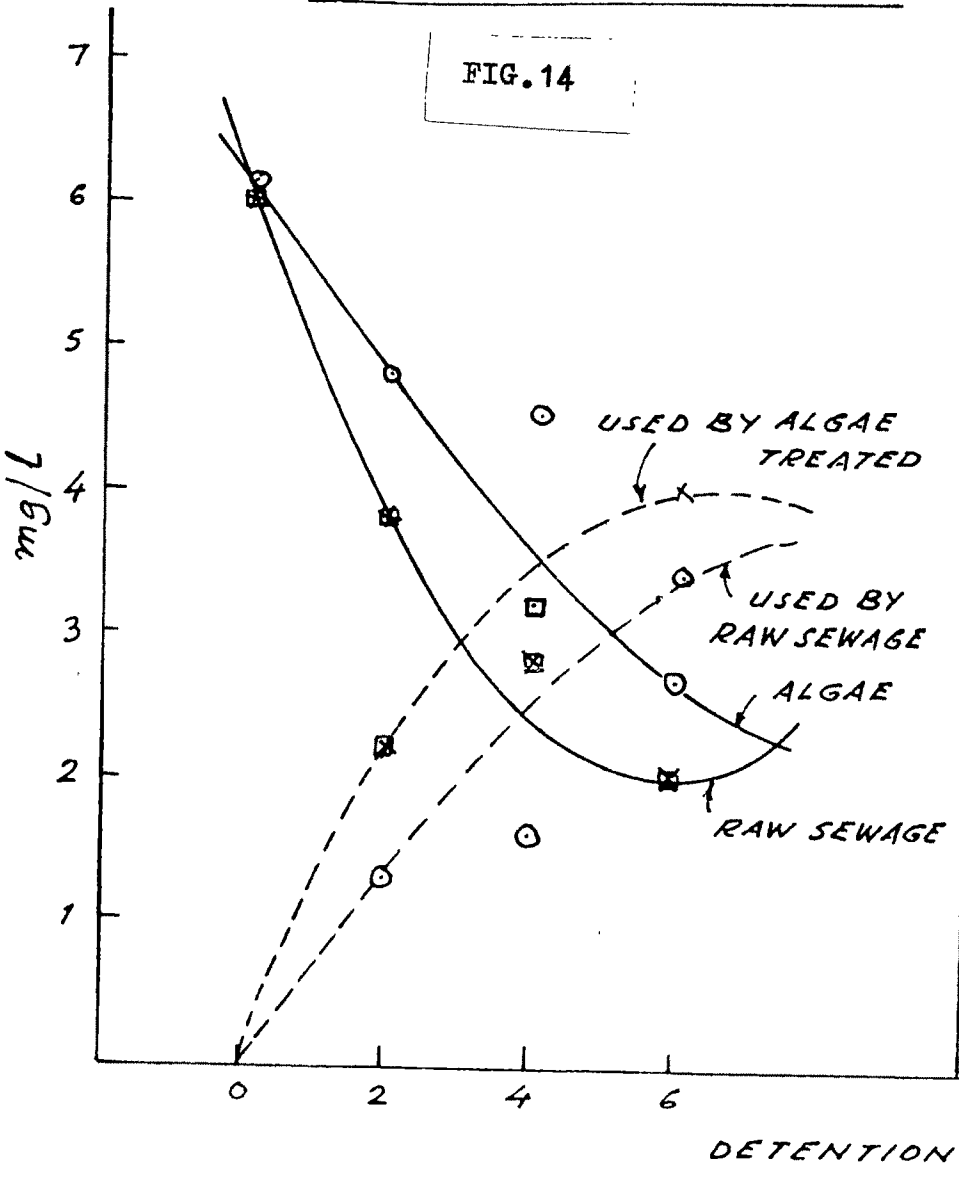
FIG. 13

TOTAL SUGAR USED AND REMAINING IN THE EXPERIMENT WITH SCENEDESMUS OBLIQUS



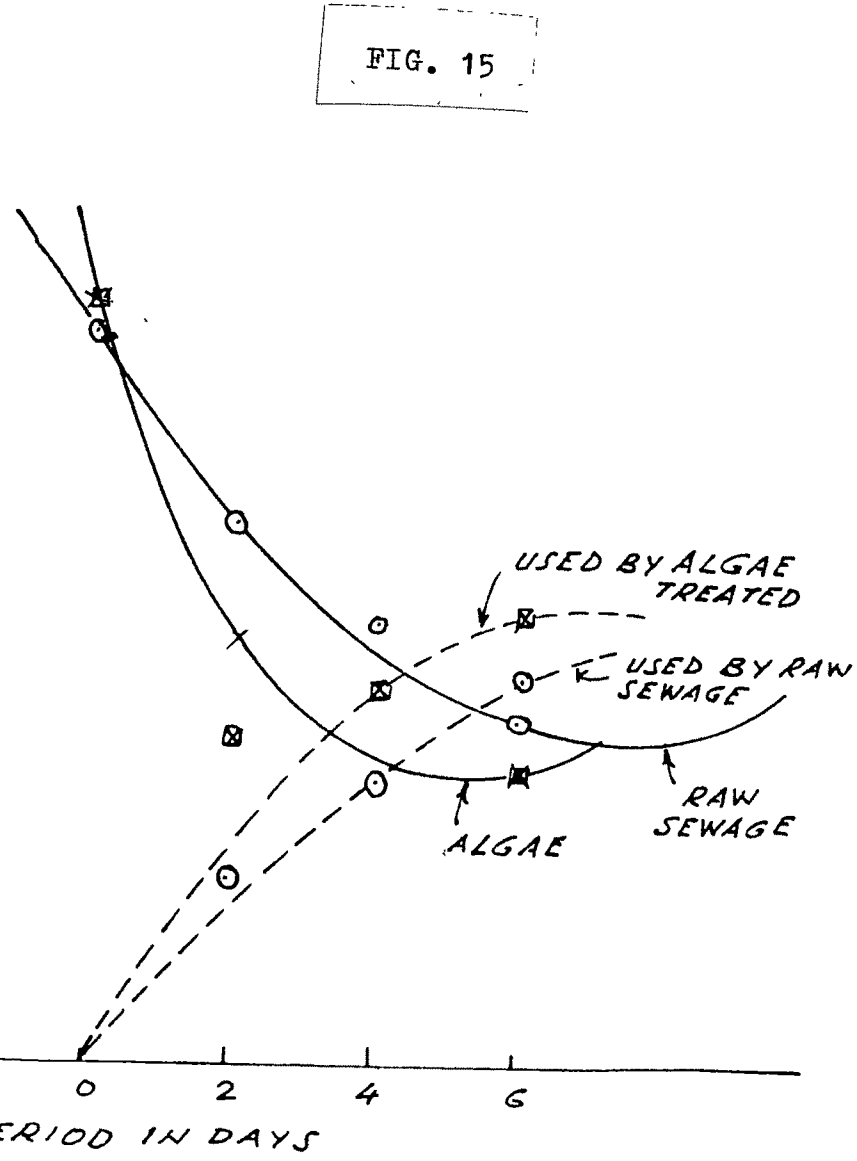
PROTEIN USED AND REMAINING IN THE EXPERIMENT WITH - SCENEDESMUS OBLIQUE

FIG. 14



AMINO-NITROGEN USED AND REMAINING IN THE EXPERIMENT WITH SCENEDESMUS OBLIQUE

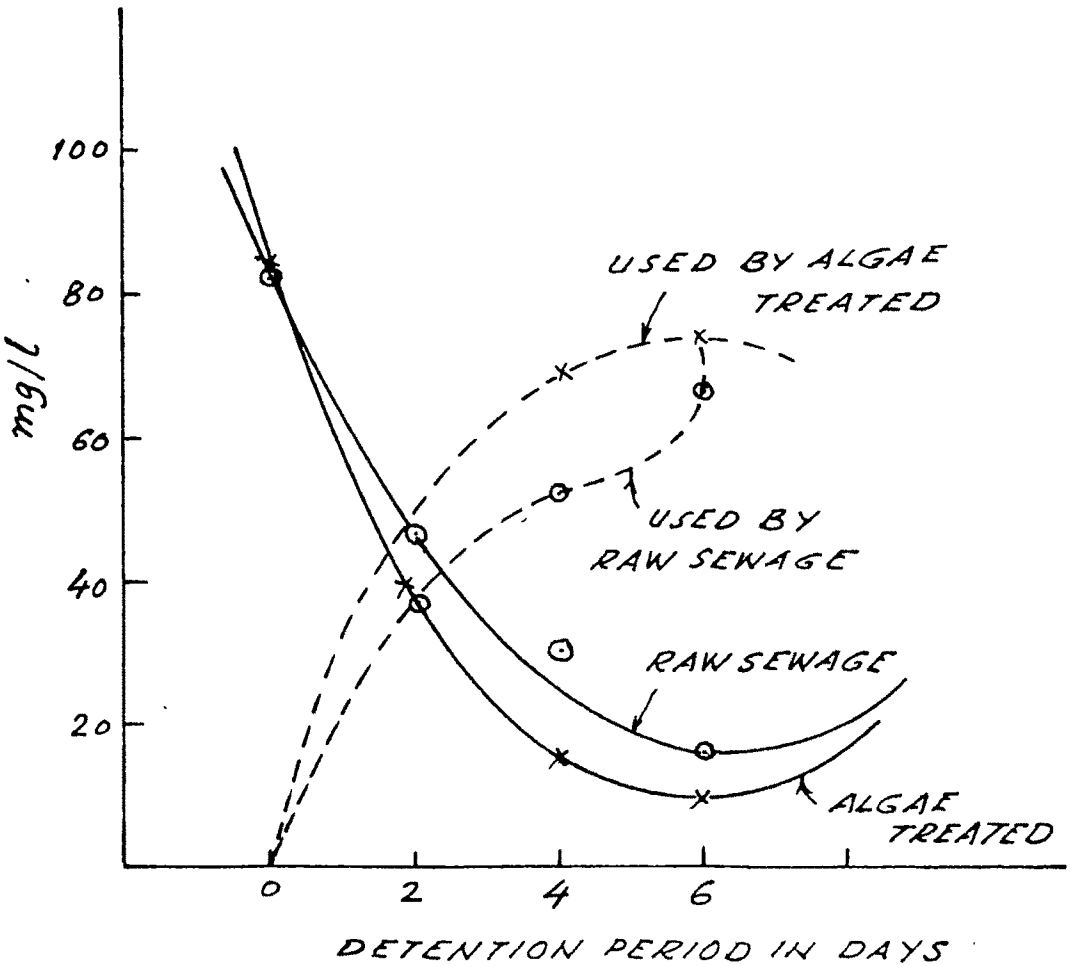
FIG. 15



DETENTION PERIOD IN DAYS

FIG. 16

VOLATILE ACIDS USED & REMAINING IN THE EXPERIMENT WITH SCENEDESMUS OBLIQUS



Applying the above formula (i), (ii) and (iii) to our results the following calculations are made:

Name of the Algae	Regression Analysis Constants "m"	"b"	Correlation Coefficient "r" "r"
Oscillatoria Spp.	+0.265	+219.6	+0.990
Anacystis nidulans	+0.159	+163.4	+0.990

There is a direct correlation between the two sets of figures.

4. Biochemical aspects of the experiment using Scenedesmus obliqus are as follows: (Fig. 12 to 16).

Percentage of reduction in the control flask within 6 days for free sugar was 55.3, total sugar 59.2 protein 55.7 amino acid nitrogen 52.9 and volatile acid 69.1. These reductions were caused by the metabolic activities of bacteria from the oxygen furnished by surface reaeration. In the case of the algae treated flasks, the reductions showed within 6 days were for free sugar 86.6%, total sugar 82.7%, protein 66.7%

amino acid nitrogen 60.3% and volatile acid 88.1%. The greater degree of reduction was found because of greater availability or production of photosynthetic oxygen for the bacterial oxidation.

5. Biological aspects of the experiment using Oscillatoria spp.

a) The presence of rotifers is an indication of the good quality of effluent. But its presence should not be encouraged as they eat away algae.

b) Brownish filaments (Fig.P.52) resembling the Iron bacterium Leptothrix ochracea are seen in fairly large numbers; and the significance of their presence is not understood.

6. Bacteriological Aspects:

1. Micro-organisms in action in Sewage purification:

The activated sludge process was developed by Ardern and Lockett in 1914 and since then it has attained such a popularity and use that no other process has attained. For more than half a century that it has

been in use, much emperical knowledge has been gained about the technical operation of the process; but unfortunately little is known still as to what effects the stabilizing action that takes place when air is diffused into activated sludge. It is no doubt the metabolic processes that are taking place then, that form the basis of purification. But the available literature reveal very little about the nature of the purification processes. Only a few basic facts have been found regarding the ecology and metabolism of activated sludge process upto the present day.

About the middle of this century Oswald, Gotaas and Golucke of the Sanitary Engineering School of the University of California developed their high-rate aerobic oxidation pond, a new low cost method of sewage purification which is based on the primorderal process of photosynthesis. Much less is known about the mechanism of purification of this later process than in the case of the older activated sludge process for, the discoverers of the process themselves state as follows:

(i) "Although reports which list the specific organisms involved in aerobic oxidation in stabilization ponds are not available, it is extremely likely

that aerobic bacteria of ponds which are mainly contained in a yellow brown flocculant sludge (the substance created during bio-flocculation) differ but little from those found in activated sludge or in trickling filter slimes (22)" (Oswald, 1960)

(ii) Oswald (1960, p. 384) has continued that " A healthy sludge comparable to activated sludge is maintained in the pond, provided mixing is carried out for about three hours a day. Following an initial accumulation, the volume of aerobic sludge does not increase, but rather remains constant indicating essentially that total oxidation is taking place".

(iii) Again Golueke (1960) has observed as follows: "An extensive knowledge of the ecology of the organisms involved in the process for the treatment of waste in high-rate oxidation pond is required. This is true because effective biological control requires an optimum relationship between the environment and the biotic community concerned, and thus can be accomplished only by providing proper environmental conditions. To establish a relationship, it is necessary to know the nature of and extent of the influence of the principal environmental factors to which an algal-bacterial community is subject in an oxidation pond..".

"There is paucity of information in the literature on the effect of these environmental factors either individually or collectively on such organisms when living as members of a biotic community".

From the foregoing three references it will be seen that (i) so far no attempt has been made to identify the specific micro-organisms which are responsible for purification in the high-rate oxidation method; (ii) that a healthy sludge comparable to an activated sludge is maintained in the pond; (iii) that the volume of the sludge does not increase but remains constant indicating (iv) that total bacterial oxidation is taking place. In short, the originators of the high-rate oxidation ponds seem to think that metabolic processes similar to an activated sludge process are taking place in two important respects: (i) in that the volume of the sludge does not increase quite unlike as in the activated sludge process; and (ii) that total bacterial action is taking place which is also not the case in the classical activated sludge process.

So, to understand the mechanism of purification in the high-rate oxidation pond it is necessary to trace briefly the basic facts known to-date about the

metabolic processes taking place in activated sludge and the micro-organisms which are known to bring about the purification since 1914, and it is done below:

2. Micro-organisms identified in activated sludge:

i) Russel and Bartow (1916) indicated 13 varieties of non-nitrifying bacteria from activated sludge. Nine of them belong to the Bacillus group of aerobic spore formers, formed acid but no gas from glucose and hydrolysed starch and casein.

ii) Kamm (1917) got almost the same results like Russel and Bartow.

iii) Buswell and Long (1923) stated that activated sludge consisted of Zoogloeal masses mixed with filamentous bacteria.

iv) Harris et al. (1927) found that 61% of the organisms in activated sludge were of Aerobacter aerogenus type and the rest of the Proteus type. The results of the earlier workers seemed to show that the Coliforms and spore formers predominated in activated sludge and thus played an important role in purification.

v) Butterfield (1935) and Winogradsky (1937) isolated Zoogloea forming organisms from activated sludge and the latter classified her strains as a Nitrocystis sp. But the former described his strain as Zoogloea ramigera. After the isolation of the organism by Butterfield in 1935, this organism was considered as the dominating organism in activated sludge until the middle of this century by several investigators (Heuklekian, Littman, Wattie, etc.), who described it as rod shaped, motile, with one polar flagellum, aerobic non-sporing Gram-negative, capsule forming; producing little or no acid from carbohydrates; producing ammonia from gelatin, casein and peptone; no nitrification; no H_2S; scant growth on agar or gelatin but producing well origanized flocs when aerated in sterile sewage.

vi) From 1937 to 1943 Butterfield, Heukelekian, Wattie and Cownkers studied the precipitation of sterile sewage using pure cultures of Zoogloea ramigera strains. Butterfield, Ruchhoft and Mc Namee (1937) found 50% BOD reduction after 5 hours aeration and 80% after 24 hours.

vii) According to Heukelekian and Littman (1939) activated sludge consisted of flocculant masses of Zoogloea ramigera.

viii) Buck and Keefer (1939) had isolated a non-motile, slide forming, Gram-negative rod with no capsule reducing nitrate to N, not affecting sugars and forming flocs under certain conditions.

ix) Butterfield and Wattie (1941) suggested that the active substance in purifying sewage in trickling filters and in activated sludge process is by Zoogloea ramigera.

x) Allen (1944) found that the majority of bacteria in the activated sludge consisted of non-spore forming rods, not affecting sugars and tentatively classified as belonging to the genera Achromobacter, Flavobacterium and sometimes to the genus Pseudomonas on account of diffusible fluorescent.

xi) Allen (1944) showed that most of the organisms in activated sludge were proteolytic, Gram-negative, rod-shaped organisms belonging to the genera Achromobacter, Flavobacterium and Pseudomonas. Coliforms were found only in smaller numbers.

xii) After the middle of this century Mckinney and Horwood (1952), Mckinney and Weichlein (1953) isolated several organisms other than Zoogloea ramigera, and

which had the ability to form flocs under suitable conditions in pure cultures. They found Escherichia coli, Escherichia intermedia, Paracolobacterium, aerogenirdes, Nocardia actinomorpha, Bacillus cereus and a number of strains of genera Pseudomonas, Alcaligenes and Flavobacterium to possess this capacity These floc forming bacteria reduced the BOD in waste water to 66-68% after 24 hours aeration.

Calaway et al. (1952) showed that the distribution of predominant species diffused with filter depth in the case of sewage filtrate through sand. The upper 12" had the greatest number and widest distribution of species. 14 species were isolated from various levels. Flavobacterium and Bacillus were predominant throughout the filter and Zoogloeal bacteria were found in high numbers in the upper 12" of sand.

xiii) Fieldman (1955) stated that Zoogloea ramigera was the main bacterial species responsible for purification in trickling filters. On account of the ability of the organism to flocculate [redacted] and to stabilize polluting organic matter, it was universally considered as the organism primarily responsible for purification in activated sludge process.

xiv) Dugan and Lundgren (1960) isolated a gram-negative rod with floc forming properties from activated sludge. This bacteria did not affect carbohydrates. These investigators did not attempt to elucidate the mechanism of the floc formation by these bacteria, and also about the role of the floc-forming bacteria in activated sludge formation under natural conditions and in stabilizing waste water (Van Gils, 1964 p.13).

xv) Van Gils (1964, p.38) found Pseudomonas to be a minor part of predominant bacteria in sewage grown activated sludge. Members of the genera Achromobacter, Alcaligenes and Flavobacterium were found to be the main constituents of the bacterial flora of such sludges. Most of the Gram-negative rod shaped strains isolated from the domestic types of activated sludge did not produce acid from glucose. A large part of these strains was not able to affect glucose at all, thus presumably belonged to the genera Alcaligenes and Lophomonas. The strains attacking glucose without production of acid apparently belonged to genus Achromobacter or if they had yellow colonies to the genus Flavobacterium. A smaller number of Gram-negative rod shaped strains utilized glucose aerobically

with production of acid, many of these strains had yellow colonies and probably were representatives of the genus Flavobacterium while of the remainder a few had a positive oxidase reaction test (Pseudomonas) and the others were considered to belong to the genus Achromobacter. Only a few Coryneforms, all belonging to the genus Arthrobacter were isolated from domestic activated sludge.

3. Micro-organisms in action in high-rate aerobic pond:

The micro-organisms isolated and identified in in the laboratory experiment are shown in Tables 7-10 (Appendix and on pages) and they are the same as those found in activated sludge process also.

7. Basic facts about the metabolic reactions taking place in an activated sludge process:

The main object of aerobic biological waste treatment is the removal of organic substances from the waste water. This is achieved by two important metabolic processes taking place in the ecosystem. They are: (a) Complete oxidation of a part of the organic substrates resulting in the formation of CO_2, H_2O and

free energy, and (b) biosynthesis and growth accompanying the decomposition of the remaining organic substrates, and newly formed cells are a major end product of this intermediate metabolism. It is on account of the latter process that the activated sludge process maintains and even increases t itself (Symons and Mckinney, 1958). So, a very striking feature of microbial metabolism in waste treatment systems generally is the relatively enormous amount of new bacterial cells which are normally produced during the oxidation of organic substrates.

One should therefore, expect to find a heavy accumulation of bacterial sludge in the high-rate aerobic pond system also. But Oswald (1960, p. 384) has stated that a healthy sludge comparable to activated sludge is maintained in the pond and that following an initial accumulation of volume of aerobic sludge does not increase but rather remains constant indicating that "total oxidation" is taking place. In our three laboratory batch culture experiments with three different types of algae, there was no accumulation of sludge as in the classical activated sludge process but only comparatively little brownish deposits

were seen intermixed with algae, when viewed under a microscope. Also, the formation of a 'constant' volume of bacterial sludge in Oswald's field ponds and very little sludge in our laboratory experiments is possible only if the system is operated on endogenous metabolism resulting in "total oxidation" of bacterial sludge.

Hoover et al. (1952) found that activated sludge rapidly removed the organic matter from solution and converted much of it into protoplasm which was degraded when all organic matter was removed. This fundamental concept was applied to Dairy waste treatment by Porges (1960) who concluded from his exhaustive studies that it should be theoretically possible to arrange conditions so as to maintain a balanced system in which sludge or bacterial cells did not accumulate. All that would be required is sufficient nutrients to produce enough cells to replace those being oxidized by endogenous respiration. He has added that this ideal state has been approached but not attained. But it would appear that this ideal state has been attained in Oswald's high-rate aerobic pond system.

Very soon however, it was found that it was impossible to burn up activated sludge completely by aeration. In the ideal case the reduction in mass of activated sludge by aerobic digestion balances the growth of new sludge so that no surplus sludge is left for disposal. From the experience in U.S.A. it is known that "total oxidation" of sludge cannot be achieved since there is always a fraction which is inert and which cannot be further broken down by aeration. Kountz and Forney (1959), and Washington and Symons (1962) found that the non-degradable portion remaining to be about 20% of the maximum mass of micro-organisms found or 11 to 15% of the ultimate BOD_5 removed. Mcwhorter and Heukelekian (1964) reported the inert matter to be 12% of the initial COD and Washington and Hetting (1965) to be about 10% of the COD consumed. So, the constant volume of sludge reported by Oswald (1960 p. 384) in the high-rate aerobic pond may consist essentially of inert matter. Further work is necessary to determine the nature of its biochemical constituents.

It would therefore seem that the high-rate aerobic oxidation pond system is operated on endogenous metabolism resulting in total oxidation, and therefore one would expect to find entirely different

types of organisms during its assimilation and endogenous phases. In fact Jasewicz and Porges 1956) and Porges (1960) have made a complete survey of the bacteria in action in a dairy waste activated sludge. They found the presence of Pseudomonas and Achromobacteriaceae, when the sludge was in endogenous phase and the presence of Bacillus and Bacterium in its assimilation phase. However these results were not confirmed by Admase (1968) in his systematic and equally thorough investigation of the bacterial flora of a similar dairy waste activated sludge. He found no significant difference in composition of the activated sludge bacterial flora before and after feeding. In our own case also, no significant difference in composition of the bacterial flora in the two phases is found, eventhough conditions favouring endogenous metabolism existed.

8. Why "total oxidation" impossible in High-rate Aerobic Ponds:

Before discussing the subject of "total oxidation" in a high-rate aerobic pond, it is considered essential to understand how a pilot plant has been worked. An account of the same is furnished below, the details having been taken largely from the two important papers

of Oswald et al. (1959) and Oswald (1960).

a) An account of the working of High-rate aerobic Pond:

Their pilot pond measured 17 x 140 ft. A polythelene sheet was then laid in such a manner as to extend over the entire area and to lap over the top of the side walls consisting of planks. The depth and detention periods were varied for the several experiments from 5" to 12" and the detention period from 2 to 4 days. The recirculation ratio was set at 8:1. Continuous feeding could not be done throughout the day. Instead, the pond was fed once each day by pumping sewage to the inlet end and simultaneously displacing an equivalent amount at the effluent end, until a volume of sewage had been added which was equal to that required to maintain the desired detention period. The volume of displacement was controlled by manual operation of the feed pump.

Hydraulic and Organic Loadings:

i. Hydraulic load (inches) = 2 - 5
ii. Organic load (lbs/acre/day) = 100 - 200
iii. Pond influent, BOD_5 at 20°C = 200 ppm.
iv. Pond effluent BOD_5 at 20°C = 20 to 30 ppm.
v. % BOD removal = 80 - 95

Sampling was done at 1 hour intervals during a 4 hours period following the introduction of sewage and at 12 hours intervals during the remainder of the 24 hours period. The results obtained during a 24 hours period are detailed below:

"A 24 hour study was conducted with a plastic lined high-rate experimental pond, the contents of which were mixed at the beginning of the study and then dosed with sewage within two hours. Transient change in the pond BOD, dissolved oxygen concentration, and rate of deposition of settleable solids were studied. About 90% of the BOD in the pond at the start of the experiment was in the bottom sludge. After mixing, about 12% of the bottom BOD remained in the supernatant and the remaining 88% quickly settled at the bottom".

"Upon adding a single dosage of sewage, a large portion of the added BOD also settled to the bottom of the pond as a sludge year. At the end of the 24 hours period, the BOD of the pond supernatant had returned to its level at the start of the study, showing that all of the dissolved BOD was treated within the period. Thus a state of equilibrium existed in the pond in which the daily increments of BOD were

disposed by a complete aerobic process".

"After mixing, the BOD of the pond liquid did not return to its previous level, because some of the oxidizable organic matter in the disturbed layer had become dissolved and was re-distributed throughout the pond by mixing. The material giving rise to this fraction of BOD probably represents the breakdown products of bacterial decomposition, which is constantly taking place in the sludge layer. Mixing serves the essential function of dispersing and aerating these breakdown products".

"The limited increase in BOD_5 of the pond liquid directly after adding sewage probably was due to the fact that sometime was required before recirculation brought about complete mixing. Moreover throughout this period portions of the newly added sewage were undergoing oxidation and bio-flocculation, so that the fraction in the supernatant decreased rapidly. That organic matter was being stored in the bottom sludge, is indicated by the fact that the total BOD bottom sludge plus pond liquid was almost four times that of the pond liquid after recovering the sewage loading. The slow decline in BOD during the 2 to 6

hours interval after adding sewage probably was a function of both, oxidation and settling. Apparently the relatively slow process of oxidation was accompanied by a built up of floc particles through bacterial activity. As the floc particles increased in size, they began to settle rapidly; hence the rapid decline in BOD during the interval between the 6th and 8th hours".

"The slow rate of decline in BOD from the 8th to 17th hours after adding sewage represents the slow oxidation and settling of organic matter resistant to rapid bacterial action. The rate of decline in BOD_5 during this interval was low because the overall concentration of organic matter in the liquid phase was low".

"About 90% of the suspended settleable solids were on the bottom of the pond within 15 minutes after completion of the mixing operation. This was about the time for the dissolved oxygen concentration to reach its level prior to mixing".

"The steady decline in dissolved oxygen concentration from the 3rd to 11th hours after mixing probably was cuased by the increased utilization of oxygen by bacteria when stimulated by the added BOD

load; and during the evening by the cessation of oxygen production by algae".

"The gradual rise in dissolved oxygen concentration which began at 11.00 p.m. was a result of [illegible] diffusion of oxygen into the pond from atmosphere. The relation of oxygen saturated to rate of diffusion from the atmosphere continued to be such that oxygen could diffuse into the pond until dawn, at which time sufficient light was present to allow oxygen to be produced by the algae. During the period of diffusion of oxygen into the pond, the BOD level was at a point at which the amount of oxygen required for bacterial activity was less than the amount of oxygen entering the pond. Had the pond been more than 5 inches in depth, or the BOD of the sewage greater than 60 ppm., the effect of atmospheric reaeration would have been less noticeable."

"In as much as the primary settled sewage added to the pond contained a negligible amount of settleable solids, the accumulation of settleable solids on the bottom of the pond was probably due to bio-flocculation. As the particles reached a "settleable" size, they sank to the bottom of the pond where they decomposed

at a rate dependent upon the availability of oxygen. The small amount of settleable solids remaining suspended in the pond liquid at the end of the study represented the incippient floc particles or particles about to settle. These were generally discharged with effluent and probably contributed significantly to the effluent BOD".

"A high-rate pond must be shallow enough to permit the entry of light almost to the bottom of the pond and it must be mixed to maintain the deposited sludge in an aerobic condition. It must be lined to permit economical mixing to prevent undue turbidity and to control the growth of emergent vegetation".

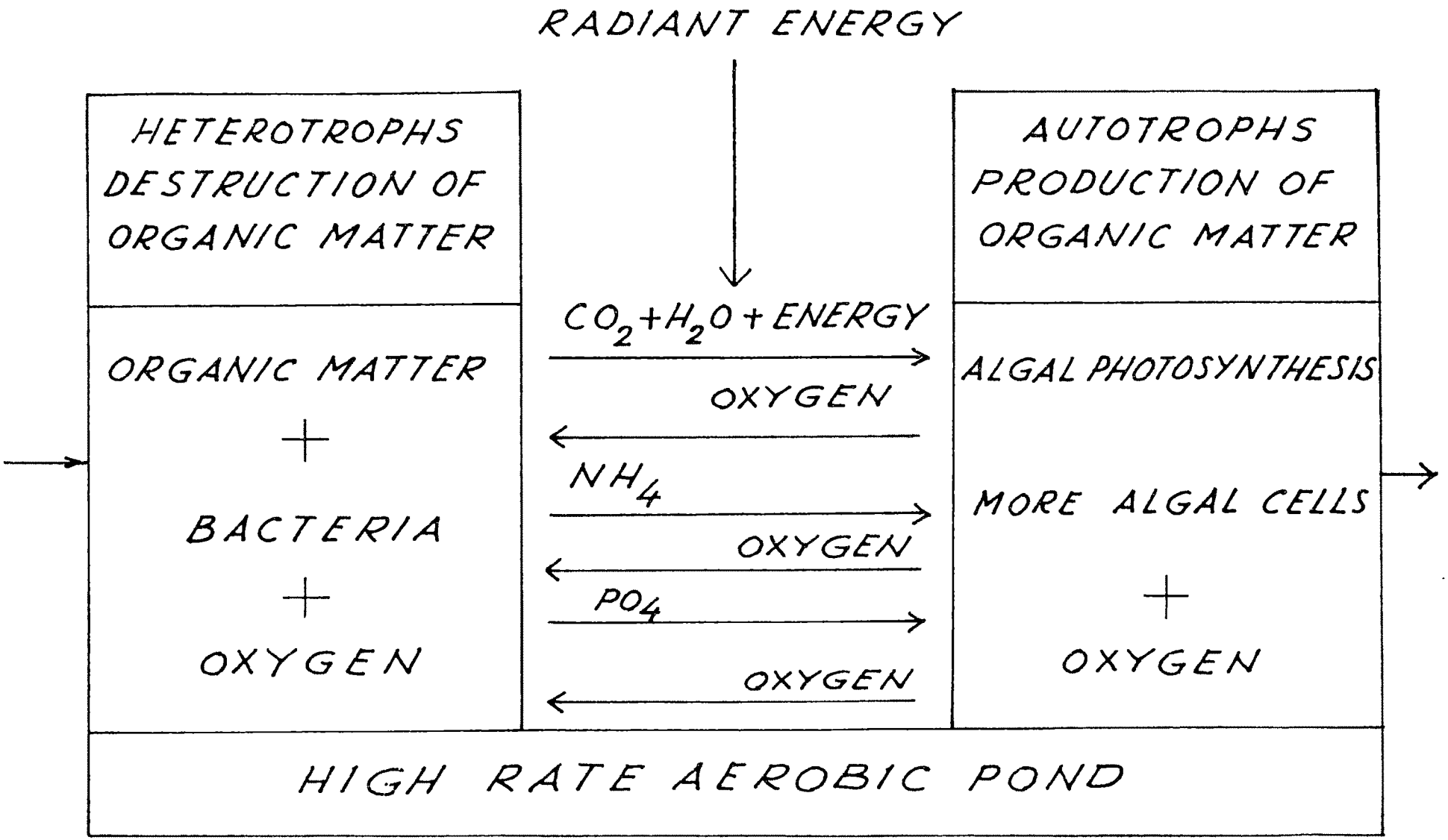
"Mixing is accomplished through the use of high-volume, low head pumps, which are automatically turned on periodically to creat a flow velocity in the pond of about 1.5ft. per second. This velocity supplies sufficient tractive force to suspend both deposited algae and deposited sludge, and to permit its contact with the oxygen rich supernatant at the pond. Experience has shown that a healthy aerobic sludge compared to activated sludge is maintained in the pond, provided mixing is carried out for about 3 hours per day.

Following an initial accumulation, the volume of aerobic sludge does not increase but rather remains constant, indicating essentially that total oxidation is taking place. During mixing, the sludge is suspended throughout the pond volume, but within 15 min. after mixing ceases, more than 80% of the sludge settles and is again distributed over the pond bottom so that sunlight entering the surface is not obscured. The algae do not adhere to the sludge nor do they become incorporated in it. Rather they remain suspended to continue their synthesis in sunlight, unless an extremely high pH brings about auto-flocculation. From the data presented in Table 4, it is evident that high-rate of entirely odour-free BOD removal may be attained in high-rate ponds".

Oswald (1960) had further added:" Although reports which list the specific organisms involved in aerobic oxidation in stabilization ponds which are mainly contained in a yellow brown flocculent sludge (the substance created during bio-flocculation) differ but little from those found in activated sludge or in trickling filter slimes (22)".

From the foregoing references it will be seen that (i) so far as attempt has been made to identify

FIG. 17


RADIANT ENERGY
HETEROTROPHS DESTRUCTION OF ORGANIC MATTER
AUTOTROPHS PRODUCTION OF ORGANIC MATTER
ORGANIC MATTER
+
BACTERIA
+
OXYGEN
$CO_2 + H_2O$ + ENERGY
OXYGEN
NH_4
OXYGEN
PO_4
OXYGEN
ALGAL PHOTOSYNTHESIS
MORE ALGAL CELLS
+
OXYGEN
HIGH RATE AEROBIC POND

the specific micro-organisms which are responsible for purification in the high-rate aerobic ponds (ii) that a healthy sludge comparable to activated sludge is maintained in the ponds; (iii) that the volume of the sludge does not increase but remains constant indicating (iv) that total bacterial oxidation is taking place. In short originators of high-rate aerobic pond seem to think that metabolic processes similar to those in a "complete oxidation" activated sludge process is taking place (Fig. 17).

b) Complete Oxidation and Constant sludge" volume in Activated Sludge process and in High-rate Aerobic Pond:

Mckinney (1962, p.231) has stated that one of the most novel processes was found in research on dairy waste by Hoover, Jasecwics and Porges (1951-1952). They found that activated sludge rapidly removed the organic matter from solution and converted much of it into protoplasm which was degraded when all the organic matter was removed. This fundamental concept which was well known in the field of bacteriology had nevern been applied to a practical problem in waste treatment. They had applied it to dairy waste treatment, Porges (1960) has concluded that " it should be theoretically possible to arrange conditions so as to maintain a balanced system

in which sludge or cells do not accumulate. All that would be required is sufficient nutrients to produce enough cells to replace those being oxidized by endogenous respiration. This ideal state has been approached but not attained". Oswald and his associates seem to have attained that "ideal state" in their high-rate aerobic pond.

c) Is total Oxidation Possible?

Rupert Kaunts and Forney (1959) at Pennsylvan State University were assigned the Job of converting the concept of "complete oxidation" from the laboratory to full scale plants. It was found that it was impossible "to burn up" the activated sludge completely by aeration. In the ideal case the reduction in mass of activated sludge by aerobic digestion balances the growth of new sludge so that no surplus sludge is left for disposal. But from the experience in USA it is known that "total oxidation" of sludge cannot be achieved since there is always a fraction which is inert and which cannot be further broken down by aeration. Kountz and Forney (1959) and Washington and Symons (1962) found that the non-degradable portion remaining to be approximately 20% of the maximum mass of microorganisms formed or 11 to 15% of the ultimate BOD_5

removed. Mcwhorter and Heukelekian (1964) reported that the inert organic matter to be of 12% of the initial COD; and Washington and Hetting (1965) to be about 10% of COD consumed.

d) A Constant Volume of Sludge in High-rate aerobic Pond:

So, from the statement of Oswald (1960, p. 384): "A healthy sludge comparable to activated sludge is maintained in the pond, provided mixing is carried out for about 3 hours a day. Following an initial accumulation the volume of aerobic sludge does not increase, but rather remains constant indicating essentially that total oxidation is taking place", it would appear that "the 'constant volume' of aerobic sludge remaining in the high-rate pond" may consist essentially of the inert matter and active bacterial mass. Further work is therefore, necessary to examine the nature and ratio of its biochemical constituents.